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Effects of Montmorency tart cherry (*Prunus Cerasus L.*) consumption on vascular function in men with early hypertension[1]

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ABSTRACT

Background: Tart cherries contain numerous polyphenolic compounds that could potentially improve endothelial function and reduce cardiovascular disease risk.

Objective: We sought to examine the acute effects of Montmorency tart cherry (MC) juice on vascular function in subjects with early hypertension.

Design: A placebo-controlled, blinded, crossover, randomized Latin square design study with a washout period of ≥14 d was conducted. Fifteen men with early hypertension [systolic blood pressure (SBP) ≥130 mm Hg, diastolic blood pressure ≥80 mm Hg, or both] received either a 60-mL dose of MC concentrate or placebo. Microvascular reactivity (laser Doppler imaging with iontophoresis), arterial stiffness (pulse wave velocity and analysis), blood pressure, and phenolic acid absorption were assessed at baseline and at 1, 2, 3, 5, and 8 h postconsumption.

Results: MC consumption significantly lowered SBP ($P < 0.05$) over a period of 3 h, with peak reductions of mean ± SEM 7 ± 3 mm Hg 2 h after MC consumption relative to the placebo. Improvements in cardiovascular disease risk factors were closely linked to increases in circulating protocatechuic and vanillic acid at 1–2 h.

Conclusions: MC intake acutely reduces SBP in men with early hypertension. These benefits may be mechanistically linked to the actions of circulating phenolic acids. This study provides information on a new application of MCs in health maintenance, particularly in positively modulating SBP. This trial was registered at clinicaltrials.gov as NCT02234648. *Am J Clin Nutr* doi: 10.3945/ajcn.115.123869.

Keywords: cardiovascular risk factors, hypertension, phenolic acids, tart cherries, blood pressure

INTRODUCTION

Cardiovascular disease (CVD)[4] is the primary cause of global mortality (1). In the United States, 1 in 4 deaths can be attributed to a cardiovascular-related event, equating to ∼610,000 people/y (2). In Europe, CVD is a major cause of death in adults and is responsible for 48% of all annual deaths (2, 3). Epidemiologic studies have suggested that polyphenol-rich foods can exert positive cardiovascular health benefits (4–6) on blood pressure (BP) (7), insulin resistance (8), cholesterol concentrations (9), and platelet activity (10), which are thought to be attributable to the high phytochemical content in fruits and vegetables (11). Several studies have investigated these cardiovascular health benefits of polyphenolic-rich foods; for example, George et al. (12–14) examined the acute and chronic effects of a fruit and vegetable puree-based drink on vascular function and other CVD risk factors and showed that endothelium-dependent vasodilation was greatest at 3 h after consumption. In addition, Dohadwala et al. (15) demonstrated improvements in arterial stiffness and brachial artery flow-mediated dilation at 4 h after cranberry juice consumption.

Montmorency tart cherries (MCs) *(Prunus cerasus L.)* and their derivatives are high in numerous phytochemicals (16–20), including the flavonoids isorhamnetin, kaempferol, quercetin, catechin, epicatechin, procyanidins, and anthocyanins (21, 22). It has previously been shown that MCs attenuate inflammation (16) and oxidative stress (23, 24) and accelerate exercise recovery (23–25). Furthermore, cherry extracts have been shown in cell and animal models to exert a range of cardioprotective effects, including increasing nitric oxide (NO) production and antioxidant status, reducing lipid oxidation, and inhibiting inflammatory pathways (16, 17). However, data from human trials are not clear. It was recently postulated that the health-related benefits associated with MC consumption might at least partly result from the downstream metabolites of the principal anthocyanins present in the fruit (20). Specifically, this work demonstrated an increase in plasma phenolic acids [vanillic acid (VA) and protocatechuic acid (PCA)]

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[4] Abbreviations used: AIx, augmentation index; BP, blood pressure; CHL, chlorogenic acid; c_{max}, maximum plasma concentration; CVD, cardiovascular disease; DBP, diastolic blood pressure; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DVP, digital volume pulse; eNOS, endothelial NO synthase; HR, heart rate; LDI, laser Doppler imaging; LC, liquid chromatography; MC, Montmorency tart cherry; MS, mass spectrometry; NO, nitric oxide; NO_x, nitrate/nitrite; PCA, protocatechuic acid; PWA, pulse wave analysis; PWV, pulse wave velocity; RI, reflection index; RS, rapid separation; SBP, systolic blood pressure; SI, stiffness index; SNP, sodium nitroprusside; t_{max}, time to achieve maximum plasma concentration; VA, vanillic acid.

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after MC consumption in humans. These specific phenolic acids are known to improve vascular function (26, 27) and have been shown to modulate vascular smooth muscle cell behavior in vitro (20); however, it is unclear whether MC consumption influences in vivo cardiovascular function. In a previous study that investigated this premise, Lynn et al. (28) examined the effect of a tart cherry juice supplement on arterial stiffness and inflammation in healthy adults and showed no positive response. The authors speculated that this was because of numerous limitations, including their sample size and blood sampling technique.

Although the potential for dietary polyphenols to improve cardiovascular health is encouraging, there is a clear need for randomized placebo-controlled trials with appropriate experimental controls to ascertain the role that polyphenols from whole foods and their analogs might exert in health maintenance. Because MCs are high in numerous phytochemicals (such as anthocyanins) that increase the bioavailability of phenolic acids in vivo, one would expect that they would positively modulate vascular function. Therefore, the aim of this study was to examine the acute effects of MC consumption on arterial stiffness, BP, and microvascular vasodilation in men with early hypertension.

METHODS

Participants

Sixteen nonsmoking men with early hypertension [systolic blood pressure (SBP) ≥130 mm Hg, diastolic blood pressure (DBP) ≥80 mm Hg, or both) volunteered to participate for this trial (NCT02234648). Baseline characteristics are presented in **Table 1**. The criterion for inclusion was a resting SBP ≥130 mm Hg. A total of 56 participants were screened before the study commenced. An SBP >120 mm Hg indicates early hypertension, reflects increased systemic vascular resistance, and is associated with an increased risk of coronary artery disease and stroke (29). Resting BP was measured with the use of an Omron 70CP automated sphygmomanometer according to British Hypertension Society guidelines after a 10-min rest. Exclusion criteria were food allergies (as discussed with the research team); history of gastrointestinal, renal, or CVD; BP-lowering or anticoagulant medication; current use of any food supplements; and other risk factors that made participants eligible for treating BP according to British Hypertension Society guidelines. All participants were otherwise in apparent good health as assessed by a health screening questionnaire. The study was conducted in accordance with the Helsinki Declaration and ratified by the Northumbria University Research Ethics Committee before participants provided written informed consent.

TABLE 1
Baseline characteristics of study participants[1]

	Value
n	15
Age, y	31 ± 9
Height, cm	182.4 ± 7.3
Mass, kg	89.7 ± 13.3
BMI, kg/m^2	27.0 ± 3.8
SBP, mm Hg	137 ± 11
DBP, mm Hg	82 ± 11
MAP, mm Hg	98 ± 11
HR, beats/min	63 ± 10

[1]Values are means ± SDs. DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

Study design

We used a randomized but counterbalanced placebo-controlled, blinded, crossover Latin square design with 2 experimental arms and a washout period of ≥14 d; participants were randomly assigned to receive either an MC concentrate followed by placebo or placebo followed by MC concentrate. A washout of ≥14 d was chosen based on a previously published study that suggested that these phenolic compounds are quickly absorbed and/or excreted (20). Each visit was at the same time of day and was preceded by an overnight fast (≥10 h). Participants reported to the laboratory at 0800 and provided a baseline venous blood sample. This was followed by baseline microvascular vasodilation assessment by laser Doppler imaging (LDI) with iontophoresis; arterial stiffness assessment by pulse wave analysis (PWA) and pulse wave velocity (PWV); digital volume pulse (DVP) and BP, all with the participant in the supine position. Participants then consumed the intervention beverage, and subsequent blood samples, LDI, PWV, PWA, DVP, and heart rate (HR) measures were taken 1, 2, 3, 5, and 8 h postconsumption. BP was assessed every hour. No food or fluid was provided during the study period except for low-nitrate mineral water. The total amount of water consumed on the first study day ad libitum was noted, and participants consumed the same quantity on the subsequent visit.

Treatments and dietary control

The MC concentrate (CherryActive) was stored at 4°C before use. Participants consumed either 60 mL MC concentrate (which according to the manufacturer is estimated to be equivalent to ∼180 whole cherries) or fruit-flavored cordial in a blinded crossover manner. The decision to use 60 mL was based on previous studies that showed a greater uptake of anthocyanin and phenolic acids in vivo after consumption (19, 20). The concentrate was diluted with 100 mL water before consumption. The placebo supplement consisted of a commercially available low-fruit (<1%) cordial (Kia Ora; Coca Cola Enterprises) mixed with water, whey protein isolate (Arla Foods Ltd.), and maltodextrin (MyProtein Ltd.) to match the MC concentrate for volume and macronutrient content (energy = 204 kcal; volume = 60 mL; carbohydrates = 49 g; protein = 2.2 g; and fat = 0 g). Before the study commenced, we explained to participants that its aim was to investigate the effect of a fruit juice on vascular function; therefore, they were unaware which beverage was the experimental drink. Participants were instructed to follow a low phenolic diet for 48 h before each arm of the trial by avoiding fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, wholemeal bread, grains, and spices and were asked to refrain from strenuous exercise. Compliance with the dietary restrictions was assessed with a self-reported standardized 2-d dietary record.

Blood sampling

Fasting whole-blood samples were collected at baseline (before supplementation) and at 1, 2, 3, 5, and 8 h in a 10-mL EDTA vacutainer system (Becton Dickinson) that was inverted to mix the anticoagulant and immediately centrifuged at 3000 × *g* for 10 min at 4°C. Plasma was aspirated into aliquots and then immediately stored at −80°C for later analysis.

LDI

Subjects were placed supine in a quiet temperature-controlled room in which the ambient temperature was 23 ± 1°C for all measures. Two perspex chambers (ION6; Moor Instruments) with an internal platinum wire electrode were placed on the volar aspect of the forearm and attached to the skin with the use of adhesive discs (MIC-1AD; Moor Instruments) and connected to the iontophoresis controller (MIC2; Moor Instruments). Acetylcholine chloride (2.5 mL, 1%; Sigma-Aldrich) in 0.5% NaCl solution was placed in the anodal chamber, and 2.5 mL 1% sodium nitroprusside (SNP) (Sigma-Aldrich) in 0.5% NaCl solution was placed in the cathodal chamber. Circular glass coverslips were placed over each chamber to prevent the loss of solutions. Current delivery was controlled by LDI Windows software version 5.1 (Moor Instruments). Skin perfusion was measured with the use of a moorLDI2-IR laser Doppler imager (Moor Instruments). The scanner head was positioned 30 cm above the chambers. The laser beam was directed by a moving mirror in a raster fashion over both chambers. A total of 20 repeat scans were taken—the first set with no current to act as a control and then 4 scans at 5, 4 at 10, 4 at 15, and 2 at 20 μÅ. The final 5 scans were measured with no current. The flux compared with time AUC over the 20 scans was calculated as a measure of microvascular response to acetylcholine (endothelium-dependent vasodilation) and SNP (endothelium-independent vasodilation).

PWV and PWA

PWV and PWA were determined with the use of arterial tonometry (SphygmoCor CPV system; ScanMed Medical). There is strong association of PWV and PWA with incident CVD that is independent of traditional risk factors (30–32). The aortic pulse waveform and augmentation index (AIx) were derived at the radial artery; PWV was determined between carotid and femoral sites. A pencil-type probe was used for all measurements and was held at the base of the neck over the carotid artery and at the inguinal crease over the right femoral artery. Recordings were taken when a reproducible signal was obtained with a high-amplitude excursion. The distance between carotid and femoral sites was measured, and electrocardiogram gating permitted the time lapse between pulse waves at the carotid and femoral sites to be calculated. The inter- and intratrial CVs for this method were 3.3% and 3.1%, respectively.

DVP

A PulseTrace PCA 2 (MicroMedical) with a photoplethysmograph transducer transmitting infrared light at a wavelength of 940 nm was placed on the index finger of the right hand and used to calculate the DVP stiffness index (SI) and DVP reflection index (RI). The DVP records the systolic and diastolic waveforms of the pulse by measuring infrared light transmission through the finger. The DVP-SI (m/s) is defined as the height of the subject divided by the time between the first and second wave peaks and is usually correlated with the stiffness of large arteries. The DVP-RI is the relative height of the second peak compared with the first and is associated with smaller artery stiffness. Collectively, these variables provide an indication of the arterial stiffness for an individual.

BP

BP was measured with the use of a noninvasive digital automatic BP monitor (M10-IT; Omron Healthcare). The BP cuff was fitted by the same researcher at each of the 9 time points. The inter- and intratrial CVs for this method were 4.6% and 2.1%, respectively. All vascular measurements took place on the noncannulated arm.

Juice analysis

Total anthocyanins

The monomeric anthocyanin pigment content of the MC concentrate and the placebo was determined with the use of the pH differential method (32). The MC concentrate was diluted 1:20 in 25 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5, respectively. The absorbance was measured with an Ultraspec UV-visible spectrophotometer (Pharmacia Biotech) at 510 and 700 nm. The absorbance difference A was calculated as $A = (A_{510}–A_{700})$ pH 1.0 $-$ $(A_{510}–A_{700})$ pH 4.5. The total anthocyanin concentration C (mg/L) was expressed as mg cyanidin-3-glucoside equivalents according to the following equation: $C = A \times MW \times DF \times 1000/(\epsilon \times l)$, where MW is the molar mass for cyanidin-3-glucoside (449.2 g/mol); DF is the dilution factor; 1000 is the conversion from grams to milligrams; ϵ is the molar extinction coefficient for cyanidin-3-glucoside (26,900 L/mol); and l is the path length (1 cm). The inter- and intra-assay CVs for this method were <5%.

Total phenolic content

Total phenolic content was measured with the use of a modified Folin-Ciocalteu colorimetric method (33). Samples were diluted in deionized water (1:10 or 1:100), and 50 μL diluted extract, 50 μL Folin-Ciocaltea reagent diluted in water (1:25), and 100 μL 6% (wt:vol) sodium carbonate were added into corresponding sample wells of a 96-well plate (Greiner Bio-One). Absorbance readings were taken at 725 nm at 5-min intervals over a 30-min period at 25°C (Synergy HT multimode microplate reader; BioTek). A stock solution of 5.8 mmol gallic acid/L was prepared in 80% (vol:vol) aqueous methanol, and quantification was performed on the basis of a standard curve in the 0–50-mg/mL range (R_2 = 0.99). The analyzed samples were measured instead of a blank sample. All values are expressed as means of gallic acid equivalents/g of sample ± SE for 6 replications. The inter- and intra-assay CVs for this method were <4%.

Trolox equivalent antioxidant capacity

A modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay used for antioxidant activity measurements was adjusted from Brand-Williams et al. (34) for use in this study. The DPPH solution was prepared freshly before analysis by dissolving the DPPH reagent (2.4 mg) in 80% methanol (100 mL), and then 10 μL extract, 40 μL deionized water, and 200 μL DPPH solution were added into each well of the 96-well plate. Absorbance readings were taken at 515 nm at 3-min intervals over a 30-min period at 37°C with a multimode microplate reader (Synergy HT; BioTek). A calibration curve with the use of 0–500 μM Trolox (R_2 = 0.99) was plotted. Final values are expressed as means of Trolox equivalents/mg of sample ± SE for 6 replications. The inter- and intra-assay CVs for this method were <3%. The total anthocyanin, total phenolic, and total antioxidant capacities for the MC concentrate and placebo are presented in **Table 2**.

Plasma analysis: HPLC

Under the selected chromatographic conditions, calibration graphs were obtained by preparing standard samples of each compound in triplicate, with increasing concentrations of each analyte. The limit of detection and linearity were calculated from calibration graphs (**Table 3**). The HPLC diode-array detector was used to identify plasma concentrations of phenolics for the acute phase of the study (presupplementation to 8 h postsupplementation). A method previously described by Bell et al. (19) was adapted for extracting phenolic compounds from the plasma. Plasma (1 mL) and 0.5 mL propyl gallate (internal standard, 50 μg; 100 μL/mL) was mixed with 4 mL oxalic acid (10 nM) and 0.1 mL HCl (12.6 M) in 15-mL falcon tubes and centrifuged at 3000 $\times$ *g* for 15 min at 4°C. The supernatant was absorbed onto a Waters Sep-Pak c17 primed (washed with 5 mL methanol with 0.2% trifluoroacetic acid followed by 2 $\times$ 5 mL water) solid-phase extraction cartridge (360 mg sorbent/cartridge, 55–105 μm). The sample was eluted with 3 mL MeOH + 0.2% trifluoroacetic acid and dried under N_2 at 45°C. Samples were then reconstituted in 400 μL 0.1% formic acid in water:2% HCl in methanol and filtered through a 0.2-μm polytetrafluoroethylene filter before HPLC analysis.

An HPLC fluorescence method for detecting and quantifying selected phenolic compounds in the plasma samples and juice was carried out with the use of a Dionex UltiMate 3000 HPLC system equipped with an UltiMate 3000 rapid separation (RS) pump, an UltiMate 3000 autosampler, and a 3000 RS fluorescence detector. The filtered samples (20 μL) were injected on a Phenomenex Luna C18(2) (250 $\times$ 2.0 mm; 5-μm particle size) reverse-phase column thermostat controlled at 30°C. The mobile phase consisted of water with 1% acetic acid (solvent A) and acetonitrile with 1% acetic acid (solvent B). After a 5-min equilibration with 20% solvent A, the elution program was as follows: 0–15 min, 20–100% solvent B (0.2 mL/min) followed by a 100% solvent B, 15–18-min (1.0 mL/min) washing stage and return to the initial conditions within 2 min. Detection was performed at the following excitation/emission wavelengths: λ_{ex} = 278 nm and λ_{em} = 360 nm for PCA and propyl gallate, and λ_{ex} = 260 nm and λ_{em} = 422 nm for chlorogenic acid (CHL) and VA, respectively. The identification and quantitation of PCA, CHL, and VA content of plasma samples was based on a combination of retention time and spectral matching of reference standards (Table 3). Samples were analyzed on a batch basis; each batch included standards prepared in 0.1% formic acid in water:2% HCl in methanol, blank control plasma samples, and fortified plasma samples at 1 (low), 10 (mid), and 25 (high) μg/mL. The recovery ranges were 88.73–94.98%, 87.31–103.78%, and 89.16–105.98% for low-, mid-, and high-fortified concentrations, respectively. The final results were collected for recovery at the low fortification concentration. Calibration curves were prepared for all antioxidant compounds, and final results are expressed as μg/mL.

TABLE 2
Total anthocyanin, phenolics, and antioxidant activity in 60 mL MC concentrate and placebo[1]

	TACN, mg cyanidin-3-glucoside/L	TPC, mean gallic acid equivalent/L	TEAC, mean Trolox equivalent/L
60 mL MC	73.50 ± 0.20	178.75 ± 0.87	0.58 ± 0.01
Placebo	ND	10.36 ± 0.13	0.01 ± 0.01

[1]Values are means ± SEMs. n = 6/analysis. MC, Montmorency tart cherry; ND, not detected; TACN, total anthocyanin content; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content.

TABLE 3
Retention times and selected UV-Vis wavelengths for quantitation of phenolics by HPLC-UV/Vis[1]

Compound	$\lambda_{ex}/\lambda_{em}$, nm	Retention time, min	LOD, μg/mL	Range of linearity, μg/mL
Protocatechuic acid	278/360	7.07	<0.01	0.05–50
Chlorogenic acid	260/422	7.65	<0.05	0.25–50
Vanillic acid	260/422	9.09	<0.01	0.05–50
Propyl gallate	278/360	11.91		

[1]LOD, limit of detection; Vis, visible; $\lambda_{ex}/\lambda_{em}$, fluorescence wavelengths.

Liquid chromatography–mass spectrometry analysis

A liquid chromatography–mass spectrometry (LC-MS) method that used the same chromatographic conditions as the HPLC diode-array detector analyses was used to confirm individual compounds in the plasma and juice samples. Briefly, LC-MS analyses were carried out on a Dionex UltiMate 3000 RS HPLC system equipped with an UltiMate 3000 RS pump, an UltiMate 3000 RS autosampler, and a QExactive Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). Electrospray ionization at both negative and positive ion modes was performed with a spray voltage of 2.00 kV and capillary temperature of 280°C. The total ion current with a range of 100–1500 *m/z* and 70,000 resolution was measured. Sample aliquots (2 μL) were injected on a Phenomenex Luna C18(2) (250 $\times$ 2.0 mm; 5-μm particle size) reverse-phase column thermostatically regulated at 40°C. The mobile phase consisted of water with 1% acetic acid (solvent A) and acetonitrile with 1% acetic acid (solvent B). The same method as applied for the HPLC analysis was carried out on the LC-MS. The identification of phenolics in the MC concentrate was verified by retention time and spectral data comparison with the corresponding reference compounds.

Plasma total nitrate/nitrite measurements

Plasma nitrate/nitrite (NO_x) measurements were carried out on plasma samples with the use of an R&D Systems Europe NO quantification kit. All reagents, standard dilutions, and samples were prepared according to the manufacturer's instructions. Plasma total NO_x determination was used as a surrogate marker of systemic NO production. The inter- and intra-assay CVs for this method were <5%. It should be noted that this measurement is not a reliable index of NO bioactivity in vivo but may be used as an indicator of NO production.

Sample size calculation

Power calculations were performed for the primary outcome: SBP. At 80% power and 5% significance, the minimum number of participants required to allow detection of a difference of 5 mm Hg (clinically relevant outcome) between the responses to the 2 intervention drinks was estimated to be 12. A total of 16 participants were recruited to allow for dropout.

Statistical analysis

Statistical analysis was performed with the use of PASW Statistics version 21.0 for Windows (IBM). All group characteristics were reported as means ± SEs unless otherwise stated. All dependent variables were analyzed with the use of a within subject, crossover design; treatment, 2 (cherry juice compared with placebo) by time, 6 (presupplement, 1, 2, 3, 5, and 8 h postsupplement) mixed-model ANOVA. Maulchy's sphericity test was used to check the homogeneity of variance for all ANOVA analyses; when necessary, violations of the assumption were corrected with the use of the Greenhouse-Geisser adjustment. Significant main effects were followed up with the use of least significant difference post hoc analysis. As a secondary analysis, the AUC was calculated by the trapezium rule, which was subtracted from the fasting value to derive the incremental AUC. Maximum plasma concentrations (c_{max}) and times to achieve maximum plasma concentrations (t_{max}) were obtained directly from the plasma concentration-time profiles. A correlation analysis was performed by with the use of Pearson's correlation coefficient to examine the relations between indexes of vascular function and appearance of phenolic metabolites in plasma. The α level for statistical significance was set at $P \leq 0.05$ a priori.

RESULTS

Sixteen hypertensive men volunteered to take part in the study, but 1 participant voluntarily withdrew after the first study day. There were no adverse events reported in response to the intervention products. All participants (n = 15) complied with the low-polyphenolic experimental diet according to the food diaries. The washout period of ≥14 d appeared to be sufficient given that the active compounds of interest were similar at baseline for both visits.

Microvascular vasodilation by LDI with iontophoresis

There was no time, treatment, or treatment × time interaction effect observed for acetylcholine (endothelium-dependent vasodilation) or SNP (endothelium-independent vasodilation). Absolute values are presented in **Table 4**. The incremental AUC (1–8 h after ingestion) for microvascular vasodilation was not significantly different between groups ($P > 0.05$). Values are presented in Table 4.

BP

SBP exhibited a significant time (P = 0.001) and treatment × time interaction effect (P = 0.003) with the MC concentrate trial. A post hoc lease significance difference test indicated that this difference occurred at 1, 2, and 3 h after supplementation in the MC group, with peak reductions of 7 ± 3 mm Hg at 2 h after MC consumption relative to the placebo. Individual responses to 60 mL MC consumption and placebo at the relevant time points are illustrated in **Figure 1**A and B. Absolute values are presented in **Table 5**. DBP showed a significant time effect (P = 0.01) but no treatment or treatment × time interaction effects. Mean arterial pressure demonstrated a significant time (P = 0.001) and treatment × time interaction effect (P = 0.01). No other vascular variables (HR, DVP-SI, DVP-RI, PWV, AIx, and AIx corrected for HR at 75 beats/min) were altered after consumption of the MC concentrate compared with the placebo treatment. The absolute values for all variables are presented in Tables 4 and 5.

Plasma nitrite and nitrate

Because of a sampling error, blood was analyzed in 13 participants. There was no time, treatment, or time × treatment interaction effect for plasma nitrate or nitrite ($P > 0.05$).

PCA, VA, and CHL

Plasma PCA (**Figure 2**A) revealed a time ($P < 0.001$), treatment ($P < 0.001$), and treatment × time interaction effect ($P < 0.001$). PCA in plasma was higher after MC consumption across all time points relative to the placebo ($P < 0.001$). For the 60-mL MC dose, the t_{max} was 1 h after consumption, yielding a c_{max} value of 2.35 ± 0.08 μg/mL. $AUC_{0–8\ h}$ values for PCA were different between the 60-mL dose and the placebo (93.7 ± 2.3 $\mu g \cdot h \cdot mL^{-1}$ and 4.2 ± 0.3 $\mu g \cdot h \cdot mL^{-1}$, respectively; P = 0.005). The presence of PCA was confirmed in plasma by comparing the experimentally determined monoisotopic molecular weights to the literature values, all of which were within ±1.5 parts per million.

Plasma VA (Figure 2B) revealed a significant time (P = 0.001), treatment (P = 0.001), and treatment × time interaction effect (P = 0.001). VA was markedly higher in plasma for ≤5 h after MC consumption than after the placebo trial. Similar to PCA, the t_{max} for VA was 1 h after consumption, yielding a c_{max} value of 0.20 ± 0.01 μg/mL. $AUC_{0–8\ h}$ values for VA were statistically significant between the 60-mL dose and the placebo (39.6 ± 2.5 $\mu g \cdot h \cdot mL^{-1}$ and 0.5 ± 0.1 $\mu g \cdot h \cdot mL^{-1}$, respectively; P = 0.026). The presence of VA was confirmed in plasma by comparing the experimentally determined monoisotopic molecular weights to literature values, all of which were within ±1.5 parts per million. The CHL concentrations in plasma after consumption were below the limits of detection in this study.

Peak plasma PCA (1 h) negatively correlated with SBP at 1 and 2 h after MC consumption (r = −0.182 and −0.131, respectively). Peak VA in the plasma (1 h) negatively correlated with SBP at 2 h after MC consumption (r = −0.095). However, these correlations were not statistically significant. There were no negative correlations, significant or otherwise, observed in the placebo trial.

DISCUSSION

To our knowledge, this study is the first to investigate the acute effects of MC consumption on arterial stiffness, BP, and microvascular vasodilation in men with early hypertension. In support of our hypothesis, this study presents new information that consuming 60 mL MC reduced SBP ≤3 h postprandially. This improvement in BP occurred at the same time points as peak increases in plasma phenolic acid uptake.

BP is a modifiable but nonetheless major risk factor for CVD (35), and diet is believed to play an important contributing factor in the advent of hypertension. Relatively small reductions (2–5 mm Hg) in BP have been reported to have an important impact on cardiovascular mortality (36). To our knowledge, this is the first study to report a positive modulation of SBP after MC consumption. Previous studies have demonstrated that other polyphenol-rich foods such as cocoa, beetroot, and grape extract

TABLE 4
Acute effects of MC juice polyphenols on vascular function[1]

							ANOVA		
	Baseline	1 h	2 h	3 h	5 h	8 h	Effect	*P*	iAUC (0–8 h)
LDI ACh, PU									
60 mL MC	1409 ± 183	2017 ± 356	1397 ± 226	1373 ± 232	1442 ± 189	1231 ± 157	T	0.060	7459 ± 348
Placebo	1671 ± 275	1647 ± 207	1178 ± 187	1242 ± 171	1168 ± 115	1180 ± 133	T × T	0.530	6415 ± 237
LDI-SNP, PU									
60 mL MC	1666 ± 206	1616 ± 283	—	1453 ± 206	1559 ± 241	1424 ± 178	T	0.240	8543 ± 209
Placebo	1891 ± 228	1847 ± 233		1552 ± 221	1636 ± 140	1690 ± 187	T × T	0.976	8475 ± 202
PWV, m/s									
60 mL MC	6.0 ± 0.2	5.7 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.8 ± 0.2	5.9 ± 0.2	T	0.029	—
Placebo	5.9 ± 0.2	5.8 ± 0.2	5.9 ± 0.3	6.2 ± 0.2	6.1 ± 0.2	6.2 ± 0.2	T × T	0.211	
AIx, %									
60 mL MC	11.0 ± 1.7	8.4 ± 1.9	9.7 ± 1.8	8.6 ± 1.9	8.4 ± 1.9	9.5 ± 2.1	T	0.582	—
Placebo	10.8 ± 2.2	10.8 ± 2.0	10.0 ± 1.8	10.4 ± 2.0	9.0 ± 2.0	10.9 ± 2.6	T × T	0.182	
DVP–SI, m/s									
60 mL MC	5.7 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	6.0 ± 0.3	5.9 ± 0.3	6.1 ± 0.3	T	0.068	—
Placebo	6.1 ± 0.3	6.0 ± 0.2	6.2 ± 0.3	6.3 ± 0.2	6.2 ± 0.3	6.3 ± 0.2	T × T	0.957	
DVP–RI, %									
60 mL MC	47.2 ± 2.8	50.1 ± 2.8	54.6 ± 3.2	55.5 ± 3.6	53.1 ± 2.9	59.0 ± 3.7	T	0.001	—
Placebo	50.6 ± 3.5	51.7 ± 3.0	58.2 ± 4.3	57.9 ± 3.7	52.9 ± 3.5	63.4 ± 3.5	T × T	0.938	

[1]All values are means ± SEMs. n = 15. There were no significant differences between placebo and cherry concentrate treatment. ACh, acetylcholine; AIx, augmentation index; DVP-RI, digital volume pulse reflection index; DVP-SI, digital volume pulse stiffness index; iAUC, incremental AUC; LDI, laser Doppler imaging; MC, Montmorency tart cherry; PU, perfusion units; PWV, pulse wave velocity; T, time effect; T × T, time × treatment interaction effect.

can have a positive effect on BP (8, 37, 38). This study is particularly noteworthy because data from prospective observational studies have shown a reduction in mean SBP of 5–6 mm Hg over a 5-y period was associated with 38% and 23% reduced risk of stroke and coronary artery disease, respectively (36). Herein, we reported peak reductions in postprandial SBP of 7 ± 3 mm Hg relative to the placebo. Previously, Lynn et al. (28) did not detect any changes in BP after tart cherry consumption in normotensive participants (~111/70 mm Hg). This discrepancy might be attributable to suggestions that the magnitude of change in the BP response is directly related to baseline BP (39), making it possible that a higher baseline BP will likely experience a greater change after an intervention. In addition, Lynn et al. (28) had little control over the supplement timing in free-living participants. It is therefore conceivable that any vasomodulatory effects from the cherries were missed. This is especially evident given that measures of vascular function were assessed after an overnight fast; this work clearly demonstrates that any positive effects are transient and return to baseline after 4 h. The magnitude of BP-lowering effects observed in this study is comparable to those achieved by a single antihypertensive drug in mildly hypertensive patients (40) and highlights the potential importance of MCs as an adjuvant in the management of hypertension. In this study, the greatest improvements in SBP occurred in association with peak plasma PCA and VA, indicating that both of these metabolites could be partly responsible for the effects observed, particularly given that these hydroxybenzoic acids have recently been shown to modulate vascular smooth muscle cell behavior in vitro (20).

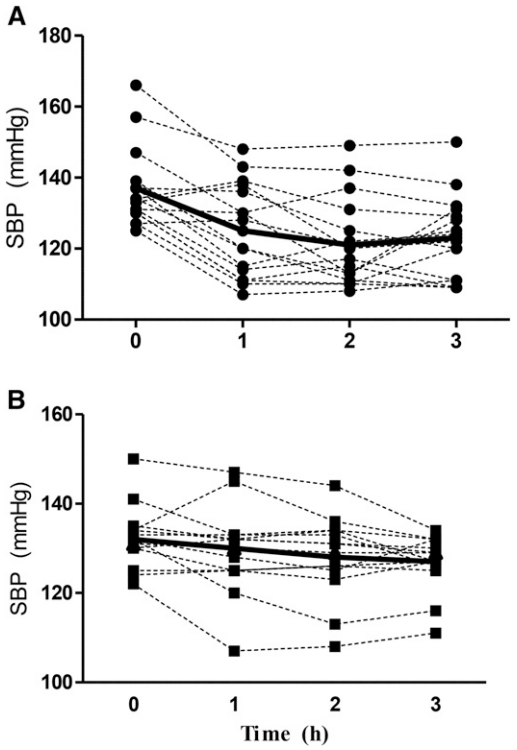

FIGURE 1 Individual responses to 60 mL MC concentrate (A) and placebo (B) consumption at relevant time points. The mean individual response is highlighted in bold. MC, Montmorency tart cherry; SBP, systolic blood pressure.

To our knowledge, only 1 study has investigated the effect of a tart cherry juice supplement on arterial stiffness (28). PWV is emerging as an important measure of vascular function that relates to CVD risk and has been shown to be a stronger predictor of arterial stiffness than AIx and central pulse pressure (41). We did not observe any significant differences in PWV between the MC concentrate and placebo across the 8-h trial. These findings are in agreement with Lynn et al. (28), in which no effect was observed on arterial stiffness in healthy subjects. Notwithstanding

TABLE 5
Acute effects of MC juice on blood pressure and heart rate[1]

										ANOVA	
	Baseline	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	Effect	*P*
PSBP, mm Hg											
60 mL MC	137 ± 3	125 ± 3[b,c]	121 ± 3[b,c]	123 ± 3[b,c]	124 ± 3[b]	125 ± 4[b]	128 ± 3[b]	128 ± 3[b]	131 ± 3[b]	T	0.001
Placebo	134 ± 2	130 ± 3[a]	128 ± 3[b]	127 ± 2[b]	127 ± 2[b]	128 ± 2[b]	129 ± 1[a]	129 ± 2[a]	131 ± 3	T × T	0.003
PDBP, mm Hg											
60 mL MC	82 ± 3	76 ± 3	75 ± 3	76 ± 2	76 ± 2	76 ± 3	78 ± 2	78 ± 2	80 ± 2	T	0.010
Placebo	79 ± 3	76 ± 3	76 ± 2	75 ± 2	76 ± 2	75 ± 2	76 ± 2	76 ± 3	81 ± 3	T × T	0.779
MAP, mm Hg											
60 mL MC	101 ± 3	92 ± 3[b]	91 ± 3[b]	91 ± 3[b]	92 ± 2[b]	93 ± 3[b]	95 ± 2[a]	95 ± 2[a]	97 ± 2	T	0.001
Placebo	97 ± 2	94 ± 2[b]	93 ± 2[a]	93 ± 2[b]	93 ± 2[b]	93 ± 2	94 ± 2	94 ± 2	97 ± 2	T × T	0.014
Heart rate, beats/min											
60 mL MC	63 ± 3	61 ± 2	60 ± 3	62 ± 3	—	62 ± 3	—	—	61 ± 2	T	0.702
Placebo	59 ± 2	60 ± 2	58 ± 2	58 ± 2		60 ± 2			61 ± 3	T × T	0.184

[1]All values are means ± SEMs. $n = 15$. [a,b]Significant difference between baseline and postintervention (1, 2, 3, 5, or 8 h), repeated-measures ANOVA: [a]$P < 0.05$; [b]$P < 0.01$. [c]Significant difference between placebo and cherry concentrate treatment, 2-factor repeated-measures ANOVA: $P < 0.05$. MAP, mean arterial pressure; MC, Montmorency tart cherry; PDBP, peripheral diastolic blood pressure; PSBP, peripheral systolic blood pressure; T, time effect; T × T, time × treatment interaction effect.

the aforementioned limitations of that study, there are several possible explanations for these results that could be applicable to our findings. Lynn et al. (28) speculated that arterial stiffness is less responsive to a short-term increase in polyphenols in healthy populations. Similar to BP, it would appear that the magnitude of change is directly related to PWV at baseline. In this study, the mean value at baseline was 5.9 m/s, which, according to the reference values for arterial stiffness collaboration (42), is well within the normal ranges for this population.

The pivotal role vascular dysfunction plays in the progression of atherosclerosis has been increasingly recognized; therefore, the vasculature has emerged as an important target for dietary therapies (43). We assessed microvascular vasodilation with the use of LDI, which measured the response to cutaneous perfusion of the forearm with acetylcholine and SNP (44). Contrary to our expectations, we did not observe any change in microvascular vasodilation after the intervention. It was somewhat surprising that no change was evident given that an increase in endothelium-dependent microvascular reactivity after high flavonoid (45) and fruit and vegetable intake (46) has been previously observed. However, these data are not in isolation; a study by Jin et al. (47) showed no change in acetylcholine or SNP response after blackcurrant consumption. However, it is particularly noteworthy that in this study certain individuals had a far higher endothelium-dependent vasodilator response than others in the same cohort. Perhaps similar to what George et al. (14) previously demonstrated, there is a differential response to the MC concentrate that is potentially based on genotype. They showed that acute consumption of a flavonoid-rich drink resulted in a considerable increase in dilation of the microcirculation in the forearm in response to acetylcholine after 180 min in GG individuals alone. However, there was no effect of the same beverage on endothelium-dependent vasodilation in the GT genotype or on endothelium-independent vasodilation in response to SNP in either genotype. However, this analysis was outside the remit of this study. These variables might be modulated after chronic supplementation and are certainly worth future investigation. In addition, the assessment of endothelial function by LDI is specific to the region examined and therefore may not provide a fuller picture of global vascular function.

Peripheral PWA is frequently used to measure the AIx, an index of arterial stiffness (48). Increased arterial stiffness results in

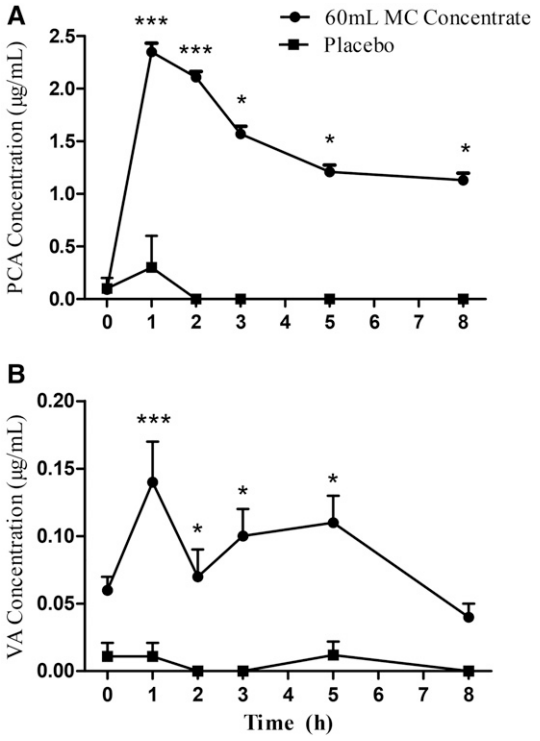

FIGURE 2 Time course of PCA (A) and VA (B) response (mean ± SEM) after consumption of MC concentrate- and macronutrient-matched control ($n = 15$). Data were analyzed with the use of a 2-factor repeated-measures ANOVA with time and treatment as the 2 factors. Significant effects of time ($P < 0.001$), treatment ($P < 0.001$), and the interaction between time and treatment ($P < 0.001$) were observed for both variables. *,***Significantly different from the placebo drink: *$P < 0.05$ and ***$P < 0.001$. MC, Montmorency tart cherry; PCA, protocatechuic acid; VA, vanillic acid.

a faster propagation of the forward pulse wave as well as a more rapid reflected wave. Therefore, a high AIx has been previously linked with greater arterial stiffness and has been shown to be a predictor of adverse cardiovascular events in a variety of patient populations (49). In this study, there were no noteworthy changes in AIx or AIx when corrected for an HR of 75 beats/min after acute ingestion of MC concentrate. Similarly, Hobbs et al. (37) failed to detect any meaningful changes in AIx after beetroot bread ingestion. Although we reported marked improvements in SBP following the consumption of MC concentrate, there were no changes in microvascular vasodilation, arterial stiffness, DVP, and HR; it has previously been reported that concurrent improvements in all measures of vascular function are not always observed (37).

There are 2 proposed mechanisms by which tart cherries are thought to improve indexes of cardiovascular function. The first is via the NO pathway, which increases the bioavailability of NO via its potential to inhibit NADPH oxidase. Cyanidin-3-glucoside, an anthocyanin found in abundance in tart cherry products (19, 22), has also been shown to increase endothelial NO synthase (eNOS) expression (50) and decrease inducible NO synthase expression (51). Such changes in the balance between eNOS and inducible NO synthase expression/abundance/activity would favor the bioavailability of the vasoactive NO. However, this study measured plasma total NO_x concentration, often used as a surrogate marker of eNOS activity (52), and found no changes in the plasma NO_x.

The second and perhaps more likely idea by which MCs improve factors associated with CVD is based on the uptake of phytochemicals that possess cardioprotective properties. An analysis of the MC juice revealed larger amounts of total anthocyanins and phenolic content than in the placebo (Table 2). To investigate a cause-and-effect relation between improvements in cardiovascular function and the intake of cherry polyphenols, we examined plasma concentrations of anthocyanin metabolite profiles after consumption. In line with BP, we observed a peak increase in plasma phenolic metabolites at 1 h after MC consumption. This also supports previous observations that showed that PCA and VA peaked between 1 and 2 h after MC consumption in healthy men (20). These phenolic acids have previously been observed in plasma after blueberry, cranberry, and blackcurrant consumption (53–55) and may be responsible for driving the beneficial vascular response observed. Both compounds have been shown to modulate vascular function in isolation (26, 27) and in concert can influence vascular smooth muscle cell behavior in vitro (20). Concentrations of these acids gradually decreased after peaking at 1 h, indicating further chemical or microbial degradation (56), excretion, or tissue uptake (57) of these compounds.

In conclusion, these data provide the first evidence to our knowledge that circulating phenolic metabolites derived from MC juice are at least partly responsible for acute improvements in SBP in men with early hypertension. This study provides further evidence that diets containing polyphenolic-rich foods have the potential to exert positive effects on vascular function. In particular, tart cherries, which contain a high concentration of bioactive phytochemicals, can modulate human physiologic function. This study also provides information on a new application of tart cherries in health maintenance, particularly in positively modulating SBP.

The authors' responsibilities were as follows—KMK, TWG, and GH: conceived and designed the research; KMK, MAB, and TC: conducted the research; KMK, CLC, and GH: analyzed and interpreted the data; KMK and GH: drafted the manuscript and had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to this study. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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