Nutrition and Disease

Consumption of Bing Sweet Cherries Lowers Circulating Concentrations of Inflammation Markers in Healthy Men and Women[1,2]

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ABSTRACT   The purpose of this study was to determine the effects of consuming sweet cherries on plasma lipids and markers of inflammation in healthy humans. Healthy men and women ($n = 18$) supplemented their diets with Bing sweet cherries (280 g/d) for 28 d. After a 12-h fast, blood samples were taken before the start of cherry consumption (study d 0 and 7), 14 and 28 d after the start of cherry supplementation (study d 21 and 35), and 28 d after the discontinuation (study d 64) of cherry consumption. After cherries were consumed for 28 d, circulating concentrations of C-reactive protein (CRP), regulated upon activation, normal T-cell expressed, and secreted (RANTES), and NO decreased by 25 ($P < 0.05$), 21 ($P < 0.05$), and 18% ($P = 0.07$) respectively. After the discontinuation of cherry consumption for 28 d (d 64), concentrations of RANTES continued to decrease ($P = 0.001$), whereas those of CRP and NO did not differ from either d 7 (pre-cherries) or d 35 (post-cherries). Plasma concentrations of IL-6 and its soluble receptor, intercellular adhesion molecule-1, and tissue inhibitor of metalloproteinases-2 did not change during the study. Cherry consumption did not affect the plasma concentrations of total-, HDL-, LDL-, and VLDL-cholesterol, triglycerides, subfractions of HDL, LDL, VLDL, and their particle sizes and numbers. It also did not affect fasting blood glucose or insulin concentrations or a number of other chemical and hematological variables. Results of the present study suggest a selective modulatory effect of sweet cherries on CRP, NO, and RANTES. Such anti-inflammatory effects may be beneficial for the management and prevention of inflammatory diseases.   J. Nutr. 136: 981–986, 2006.

KEY WORDS: • C-reactive protein • nitric oxide • phenolic compounds • blood lipids • arthritis

Cardiovascular disease (CVD)[4] and stroke continue to be the top killers in the United States, accounting for >38% of all deaths; >70 million Americans have some form of CVD (1). Major risk factors for CVD include dyslipidemia (elevated total and LDL cholesterol, elevated triglycerides, low HDL cholesterol), increased plasma C reactive protein (CRP, a marker of inflammation), homocysteine, thrombotic activity, and hypertension (1).

Numerous epidemiological studies indicate an inverse association between fruit and vegetable intake and the risk for CVD and ischemic stroke (2,3). In addition to providing essential vitamins, minerals, and dietary fiber, fruits contain polyphenols that exhibit antioxidant, anti-inflammatory, and lipid-lowering properties (4–6). Both sweet and tart cherries are rich in phenolic compounds, including anthocyanins (responsible for red skin and flesh color), catechins, chlorogenic acid, flavonal glycosides, and melatonin (7). Anthocyanins extracted from cherries exhibit anti-inflammatory properties, via inhibition of cyclooxygenase activities (8) and scavenging of the reactive NO radical (9). Anthocyanins, flavonols, and isoflavones, but not chlorogenic acid inhibited NO production in activated macrophages (10); anthocyanin-rich crude extracts from berries also inhibited NO production in a manner proportional to their phenolic and anthocyanin contents. On the other hand, the effects of phenolic compounds on TNF-α secretion by activated macrophages varied (11). Thus, the effects of different flavonols and isoflavones ranged from stimulation to inhibition, whereas chlorogenic acid had no effect; anthocyanin-rich extracts from berries increased TNF-α secretion. Phenolic compounds extracted from tart cherries and virgin olive oil provided protection against adjuvant-induced arthritis in animal models (12,13). Cherry consumption relieved symptoms of arthritis in a

preliminary human study (14). A single bolus of Bing cherries administered to healthy women after a 12-h fast tended to reduce the circulating concentrations of CRP and NO within 3 h of the bolus, although the results were not significant ($P <$ 0.1) (15). In that study, plasma uric acid was decreased, but vitamin C and the total lipophilic oxygen radical absorbing capacity were increased. Together, these reports suggest that cherries exhibit anti-inflammatory effects.

Consumption of foods rich in phenolic compounds, such as red wine, elderberry juice, lyophilized grape powder, and chocolate increased plasma HDL cholesterol and decreased LDL cholesterol and triglycerides in human volunteers (16–19). The reduction in plasma LDL cholesterol and triglyceride in these studies was postulated to be due to reduced secretion of apolipoprotein B-100 and VLDL packaging (16,19). We are not aware of any published report that has examined the effects of long-term consumption of cherries on plasma markers of inflammation and lipid profile. Therefore, the purpose of this study was to examine the effect of consuming Bing sweet cherries for 28 d on plasma markers of inflammation and lipids. We determined concentrations of serum CRP, plasma NO, IL-6, and 42 other markers of inflammation (cytokines, growth factors, chemokines, adhesion molecules, and their receptors). For blood lipids, we determined the total, LDL, and HDL cholesterol, triglycerides, and the particle size and concentrations of various subclasses of VLDL, LDL, and HDL. Clinical chemistry and hematology panels were performed to evaluate the effects of cherries on general health.

SUBJECTS AND METHODS

Subjects and study design. The Human Subjects Review Committee of the University of California, Davis, approved the study. All subjects gave informed written consent before entering the study. It was a 64-d study with 3 metabolic periods, a baseline period of 8 d (d 0–7), a cherry intervention period of 28 d (d 8–35) and a postintervention period of 28 d (d 36–64). The clinical portion of the study was conducted in June-August, 2003, during California's fresh cherry season, at the USDA Western Human Nutrition Research Center, University of California Davis. Candidates recruited from the Davis, CA area were screened for good health by a medical history questionnaire, physical exam, and standardized blood tests including a complete blood cell count with leukocyte differential, clinical chemistry panel, lipid panel, and tests for infectious disease. Candidates were excluded if they were in poor health, obese (BMI > 30 kg/m²) or used nutritional supplements, medications, alcohol, or recreational drugs on a regular basis. A total of 20 healthy subjects (2 men and 18 women) were selected to participate in this study. Data from 2 women were excluded because one had to take antibiotics for an infection during the study, and the other had CRP concentrations that ranged from 1 to 31 mg/L. Subjects were 45–61 y old (mean ± SEM = 50 ± 1) with a BMI of 20–30 kg/m² (mean ± SEM = 26.3 ± 0.9). They were advised not to change their activity level and diet except to replace an equivalent amount of dietary carbohydrates with carbohydrates from cherries during the 28 d of cherry consumption. They were also instructed to limit the consumption of foods rich in polyphenols (berries, tea, wine, grape products, fruit juice, apples, and broccoli) throughout the study. Dietary records (24 h) were collected 3 times during the study and analyzed by the Nutrition Data System for Research (University of Minnesota). Bing cherries were brought from Grower Direct Marketing, LLC, Stockton, California, on June 2, 9, and 16, 2003, to the Postharvest Pomology Laboratory at UC Davis. The samples were sorted to discard cracked, decayed, and unripe fruits, weighed into aliquots of ~45 cherries each (300 g, equivalent to 280 g when pitted at the time of consumption), placed in Zip lock bags, and kept at 0°C until distribution. These storage conditions did not freeze the cherries because the highest freezing temperature for cherries is −2°C. The cherries provided ~216 kcal or 903 kJ/d, which represented ~11% of the daily energy intake.

Sample collection. Blood samples from subjects who had fasted for 12 h were drawn by venipuncture into tubes containing EDTA or no anticoagulant on study d 1 and 7 (baseline), 21 and 35 (intervention), and 64 (postintervention). Serum and plasma samples were prepared and stored frozen at −70°C for later analysis.

Laboratory methods. Because of the high cost of some of the test reagents, not all analyses were performed on blood samples drawn on each of the 5 d. Clinical chemistry panels on blood samples were performed using Beckman Synchron Lxi 725 (Beckman Coulter). A complete cell count was done using a Cell Dyn 3200 Cell Counter (Abbott Laboratories). Serum insulin concentration was determined using an immunometric assay (DPC).

Inflammatory makers. Concentrations of serum CRP were measured by a highly sensitivity immunometric assay (DPC) and those of plasma IL-6 and TNF-α by immunoassay kits (R&D Systems). The plasma concentration of NO was calculated by measuring its oxidation products, nitrite and nitrate, using the Total Nitric Oxide colorimetric assay kit (R&D Systems). In addition to the serum and plasma markers of inflammation (CRP, IL-6, NO), ex vivo secretions of IL-6 and TNF-α were also examined in whole blood cultures stimulated with lipopolysaccharide (LPS) (0, 2 and 10 mg/L) for 24 h. Plasma concentrations of 42 inflammatory markers (cytokines, receptors, growth factors, chemokines, and adhesion molecules) were determined using inflammatory antibody arrays III and 3.1 (Ray Biotechnology) (20). Concentrations of inflammatory markers were quantified by determining their optical densities with a UVP Chemi-imager, and are expressed as pixels of optical density. Because of the high cost, this analysis was performed on only 9 pooled plasma samples, 3 each on study d 7, 35, and 64. Each plasma pool consisted of an equal volume of the plasma for the same 6 subjects on each of these study days (Pools 1, 4 and 7 included subjects #1–6 for study d 7, 35, and 64, respectively; pools 2, 5, and 8 represented subjects #7–12 for study d 7, 35, and 64, respectively. Similarly pools 3, 6, and 9 represented subjects #13–18 for the 3 metabolic study periods. Although this approach decreased the df, it also reduced the subject-to-subject variation. Differences that are significant when $n = 3$ are likely to be significant at a lower P-value for $n = 18$.

Blood lipids. Particle sizes of VLDL, LDL, and HDL, and their subfractions were analyzed by NMR techniques (21). Serum concentrations of total and HDL cholesterol and triglycerides (TG) were analyzed using automated enzymatic methods (22–24). LDL cholesterol concentration was then calculated using the Friedewald equation (25).

Phenolic compounds in cherries. Phenolic compounds were extracted from 5 g of frozen cherries with 10 mL of water:methanol (2:8) containing 2 mmol/L NaF. After filtration through a 0.45-μm filter, the supernatant was analyzed for polyphenols by HPLC with UV diode-array detection (26). Total phenolics were determined in the polyphenol extract using a modified spectrophotometer Folin-Ciocalteu method (27). Ascorbic and dehydroascorbic acids were extracted from cherries with a citric acid buffer and determined by HPLC with UV diode-array detection (28). The soluble solids content of the cherries was measured using a refractometer (Abbe refractometer model 10450, American Optical). The soluble solids consisted of sugars, organic acids, amino acids, soluble pectins, phenolic, and other soluble compounds.

Statistical analysis. The SAS PROC MIXED was used to fit a repeated-measures model with a first-order autoregressive covariance structure among the repeated measures (29). The fixed effect was day and the random effect was subject. One-tailed tests for single df contrasts were used to compare the treatment periods. Values are means ± SEM. Differences were considered significant at $P < 0.05$.

RESULTS

Chemical analysis of cherries. Total soluble solids were 18.3% of the fresh weight of cherries (well above the minimum ripeness at harvest index of 16%), and total phenolics comprised 134.4 mg/100 g cherries (**Table 1**). Hydroxycinnamates comprised the largest class of phenolics with 72.2 mg/100 g

TABLE 1

Phenolic compounds, vitamin C, and total soluble solids in Bing sweet cherries[1]

Variable	Unit/100 g fresh weight	% Phenolic compounds
Hydroxycinnamates, *mg*	72.2 ± 7.6	53.5
Anthocyanins, *mg*	35.6 ± 5.2	26.4
Procyanidins, *mg*	21.3 ± 7.8	15.8
Flavanols, *mg*	5.9 ± 0.8	4.3
Total phenolics, *mg*	135.0 ± 18.9	100.0
Dehydroascorbic acid, *mg*	17.6 ± 1.2	
Total soluble solids, %	18.3 ± 0.4	

[1] Values are means ± SEM, $n = 3$ batches of cherries.

fresh cherries or 53.6% of total phenolics; neochlorogenic acid was the major hydroxycinamate with 67.3 mg/100 g cherries. Anthocyanins were the second highest class of phenolic compounds with 35.6 mg/100 g cherries (26.4% of total phenolic compounds). These were comprised largely of cyaniding-3-rutinoside (33.1 ± 7.2 mg/100 g) and cyaniding-3-glucoside (2.3 ± 0.4 mg/ 100 g). Procyanidins, mainly catechin (14.5 ± 3.2 mg/100 g cherries) and epicatechin (5.8 ± 8.7 mg/100 g), made up 15.8% of the total phenolics. Flavanols, mainly rutin (5.9 ± 1.3 mg/ 100 g), made up only 4.3% of the total phenolic compounds. Only dehydroascorbic acid, the oxidized form of vitamin C, was detected in the cherries. No HPLC peaks were detected for the reduced form, ascorbic acid.

Assessment of dietary intake. The mean energy intake during the stabilization period was 8118 ± 615 kJ/d; the energy contribution from carbohydrates, fat, and protein was 50.4, 32.6, and 16.2%, respectively (**Table 2**). Saturated, monounsaturated, and polyunsaturated fatty acids represented 10.5, 12.3, and 7.1% of the total daily energy intake. Intake of total energy and its distribution among carbohydrates, fat, and protein did not differ during the intervention and postintervention periods compared with the corresponding values during the stabilization period.

Effect of cherries on markers of inflammation. Serum CRP concentrations did not differ between the 2 baseline determinations made on study d 0 and 7 (**Fig. 1 A**). Cherry consumption for 14 and 28 d decreased the CRP concentrations by 8 ($P = 0.11$) and 25% ($P < 0.05$) respectively, compared with baseline. The CRP concentration 28 d after the discontinuation of cherry intake (d 64) tended to be greater (10%; $P = 0.11$) than at d 35. Plasma concentrations of IL-6 on study d 7,

35, and 64, were 1.10 ± 0.35, 0.96 ± 0.18, and 0.98 ± 0.14 ng/L, respectively and were not affected by the intake of cherries. Ex vivo secretion of IL-6 and TNF-α by whole blood cultures stimulated with LPS did not change during the study (not shown). Plasma concentrations of NO were 18% lower on both study d 21 ($P = 0.04$) and 35 ($P = 0.07$) compared with the concentration on d 7 (Fig. 1 *B*). Cherry consumption reduced circulating concentrations of CRP and NO in 12 of the 18 subjects; the concentrations were not affected in the remaining 6 subjects (data not shown). Thus, there were individual variations in the responsiveness to the consumption of cherries.

After subtracting the background or the negative control, the signals generated for most of the plasma markers of inflammation determined by the protein array assay remained within the variability of the basal level; hence these readings could not be relied upon to estimate their concentrations (not shown). These low concentrations of inflammatory markers may reflect the fact that the participants in this study were healthy subjects. Four inflammation markers [intercellular adhesion molecule-1 (ICAM-1), IL-6 soluble receptor (IL-6 sR), regulated upon activation, normal T-cell expressed and secreted (RANTES), and tissue inhibitor of metalloproteinases-2 (TIMP-2)] had signal densities 5- to 10-fold greater than that of the negative control. Plasma concentrations of ICAM-1, IL-6 sR, and TIMP-2 did not differ on study d 7, 35, 64 (not shown), whereas those of RANTES decreased by 21% after 28 d of cherry consumption compared with the presupplementation concentration (Fig. 1 C, $P < 0.05$). The RANTES concentration continued to decrease even during the postsupplementation period; it decreased by 36% on study d 64 compared with d 7 (d 7 vs. d 64 $P = 0.001$). Thus, cherry consumption decreased the circulating concentrations of CRP, NO, and RANTES; concentrations of RANTES continued to decline even after 28 d of discontinuing the consumption of cherries, whereas those of CRP ($P = 0.20$) and NO ($P = 0.25$) showed a partial reversal of the reduction caused by cherries.

Effect of cherries on blood lipids, chemistry and hematology panels. Plasma concentrations for total, LDL, and HDL cholesterol, TG, and the total:HDL cholesterol ratio did not differ on study d 7, 35, and 64 (data not shown). The particle sizes for VLDL, LDL, and HDL, and the concentrations of their subfractions were not affected by consumption of cherries. Fasting blood glucose concentrations for study d 7, 35, and 64 were 4.78 ± 0.09, 4.78 ± 0.07, and 4.71 ± 0.11 mmol/L, respectively; corresponding insulin concentrations for these days were 45.84 ± 4.86, 49.31 ± 5.56, and 50.00 ± 6.95 pmol/L. Serum concentrations of Na, K, Ca, Cl, CO_2, blood urea nitrogen,

TABLE 2

Estimated macronutrient intake by study subjects before, during and after cherry consumption[1]

Nutrient	Pre-cherries Study d 7	End cherries Study d 35	Post-cherries Study d 64
Energy, *kJ/d*	8117 ± 614	7741 ± 535	7746 ± 535
Protein, *en %*	16.2 ± 0.9	14.0 ± 0.8	16.1 ± 0.7
Carbohydrate, *en %*	50.4 ± 2.6	55.4 ± 2.6	53.0 ± 2.8
Fat, *en %*	32.6 ± 1.9	32.0 ± 2.0	31.4 ± 2.2
Saturated fatty acids, *en %*	10.5 ± 1.0	10.9 ± 0.8	11.3 ± 0.9
Monounsaturated fatty acids, *en %*	12.3 ± 0.7	11.7 ± 1.1	11.7 ± 1.2
Polyunsaturated fatty acids, *en %*	7.1 ± 0.6	6.9 ± 0.7	5.9 ± 0.5

[1] Values are means ± SEM, $n = 18$. Total as well as the percentage of energy (en %) intake from different nutrients did not differ during the 3 phases of the study (repeated-measures ANOVA).
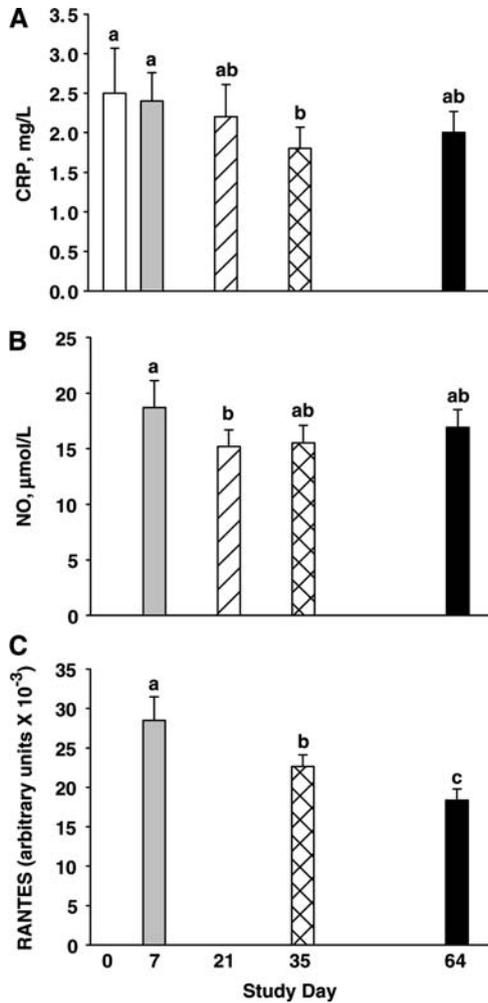
FIGURE 1 Effect of consuming Bing sweet cherries on circulating concentrations of CRP (*A*), NO (*B*), and RANTES (*C*) in healthy humans. In *panels A* and *B*, values are means ± SEM (*n* = 18). In *panel C*, *n* = 3 pooled plasma samples from 18 subjects (6 subjects/pool).

protein, albumin, creatinine, bilirubin, alkaline phosphatase, and aspartate aminotransferase were all within the normal ranges for healthy subjects and did not differ on study d 7, 35, and 64 (data not shown). Similarly, the hematological variables including platelet, red and white blood cell numbers, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, mean platelet volume, and differential cell count for neutrophils, lymphocytes, monocytes, eosinophils, and basophils were within normal ranges and did not change during the study (data not shown).

DISCUSSION

The purpose of our study was to examine the effects of consuming Bing sweet cherries on circulating concentrations of markers of inflammation and lipids. Our results showed a reduction in the concentrations of some markers of inflammation, but none of the lipid variables were altered by the intake of cherries. The serum concentration of CRP decreased by 8 and 25% within 14 d and 28 d, respectively, of consuming cherries. (Fig. 1A). A maximum reduction of 18% in the plasma concentration of NO was attained within 14 d of consuming cherries (Fig. 1B), and that concentration was maintained for

the next 14 d. After the discontinuation of cherry consumption, both CRP and NO concentrations tended to recover (*P* > 0.2) the reductions caused by cherries and did not differ from either their pre- (d 7) or post-cherry (d 35) concentrations. Because the concentrations of CRP and NO after the discontinuation of cherry intake were approximately midway between their pre- and postsupplementation concentrations and did not differ from either, we suggest that there was a residual effect of the cherry intake. Plasma RANTES concentration decreased by 21% after 28 d of cherry supplementation (Fig. 1C); however, its concentration continued to decrease even after the discontinuation of the cherries for 28 d. Thus, 28 d after stopping the consumption of cherries, the circulating concentrations of 2 of the markers of inflammation started to increase, whereas that of the third marker continued to decline. The difference in response among these 3 markers of inflammation after the discontinuation of cherries may be due to the differences in the half-lives of the cells that produce them and/or the involvement of different mechanisms such as gene activation vs. signal transduction. CRP is expressed mainly by hepatocytes, but it is also produced by macrophages and smooth muscle cells (30–32). The circulating concentration of NO is determined largely by the stimulation or inhibition of inducible nitric oxide synthase (iNOS) in macrophages (33). Activated T cells comprise the major source of circulating RANTES, although it is also produced by monocytes, epithelial cells, and dermal fibroblasts (34). Thus, the longer half-life of T cells compared with those of macrophages may be the basis of the continued decline in plasma RANTES after the discontinuation of cherries. It is notable that cherry consumption reduced serum CRP but not IL-6. These results suggest that the effects of cherries on serum CRP may not be mediated through altered secretion of IL-6. This interpretation is consistent with the results from IL-6 knockout mice showing that not all acute phase responses are mediated through IL-6 (35). Concentrations of a number of other markers of inflammation were not altered by the consumption of cherries. This may be due to the low concentrations of these markers in the healthy participants of this study, low sensitivity of the assay methods used, or the limitation of the effects of cherries to specific inflammatory pathways. Studies in subjects with elevated blood concentrations of inflammatory markers, the use of more sensitive methods, and in vivo or ex vivo challenges may detect the effects of cherries on other markers of inflammation.

The reductions in serum CRP and plasma NO by the intake of cherries in the present study are consistent with what we reported previously with an acute bolus of cherries (15). Elevated serum CRP is one of the most important indicators of inflammation and it is a significant risk factor for CVD (36). Reduction in plasma CRP by cherries can be viewed as a reduction in inflammation that may affect the risk for CVD. This is supported by the simultaneous reductions in the circulating concentrations of NO and RANTES. Increased production of NO by the iNOS and an increased plasma NO concentration were implicated in tissue injury in a variety of rheumatic diseases, including systemic lupus erythematosus, rheumatoid arthritis, and osteoarthritis (37,38). Conversely, the production of NO by endothelial NOS (eNOS) may serve as a protective or anti-inflammatory function by preventing the adhesion and release of oxidants by activated neutrophils in the microvasculature. The micromolar concentration of plasma NO in our study suggests that the reduction in plasma NO by the feeding of cherries resulted from inhibition of iNOS activity or increased clearance of NO, and was not due to the inhibition of eNOS or neuronal NOS (nNOS); constitutive expression of eNOS and nNOS yields only pico- to nanomolar plasma

concentrations of NO (37). Anthocyanins inhibited iNOS activity of stimulated macrophages in vitro (9), whereas *trans*-resveratrol stimulated and tannin inhibited eNOS activity of cultured endothelial cells (39,40). Plasma levels of nitrotyrosine (an NO-derived oxidant) are elevated in arthritis (41) and are positively associated with the risk for CVD (42). Reduction in plasma NO by cherries may have beneficial effects on the immune system and reduce the risk for CVD and arthritis.

RANTES is chemotactic for T-cells, human eosinophils, and basophils, and increases the adherence of monocytes to endothelial cells (43). Its plasma concentrations increase in several inflammatory diseases, including osteoarthritis, Sjogren's syndrome, and systemic lupus erythematosus (44–48). A reduction in plasma concentration of RANTES by cherries may signify a downregulation of the inflammatory pathways involved in such diseases.

In the current study, cherry consumption did not alter the blood lipids, glucose, and insulin levels. Results regarding the effects of phenolic compounds from cherries on blood lipids are consistent with those of a human study with elderberry juice in healthy subjects (17), but not with those of a grape powder study in obese subjects (19). It is possible that the effects of phenolic compounds are determined by the preexisting blood lipids and other obesity-related metabolic changes.

Our study design did not include a placebo group, and the markers of inflammation that were reduced by cherries did not fully return to their original levels within 28 d of discontinuing the consumption of cherries. It is possible that factors other than the consumption of cherries may have contributed to the changes observed. Because these changes were dependent on the duration of time for consuming cherries, they were most likely the result of consuming cherries. Partial recovery of CRP and NO after the discontinuation of cherry consumption again suggests that cherries caused these effects. Our results are consistent with those reported with phenolic compounds in animal models (12,13), preliminary human studies with cherries (14,15), and in vitro studies with phenolic extracts from cherries (7–11). Considering the healthy state and small number of subjects in our study, the results are quite promising. These should be confirmed in studies with larger number of subjects and also in subjects with preexisting inflammatory conditions such as CVD, arthritis, smokers, and older subjects. Because fresh cherries have limited availability, studies with cherry juice, canned cherries, cherry powder, or other fruits with similar phytochemical profile may be useful. Further studies are also warranted to determine the health effects of individual phenolic compounds found in cherries.

In conclusion, supplementing the diets of healthy men and women with cherries reduced the serum/plasma concentrations of some markers of inflammation, whereas circulating concentrations of many other markers of inflammation, lipids and their subfractions, and particle sizes were not affected. The anti-inflammatory effects of cherries may be of clinical significance and should be investigated in further studies.

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