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Sweet Bing Cherries Lower Circulating Concentrations of Markers for Chronic Inflammatory Diseases in Healthy Humans[1–4]

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Abstract

A limited number of studies have demonstrated that some modulators of inflammation can be altered by the consumption of sweet cherries. We have taken a proteomics approach to determine the effects of dietary cherries on targeted gene expression. The purpose was then to determine changes caused by cherry consumption in the plasma concentrations of multiple biomarkers for several chronic inflammatory diseases in healthy humans with modestly elevated C-reactive protein (CRP; range, 1–14 mg/L; mean, 3.5 mg/L; normal, <1.0 mg/L). Eighteen men and women (45–61 y) supplemented their diets with Bing sweet cherries (280 g/d) for 28 d. Fasting blood samples were taken before the start of consuming the cherries (study d 7), 28 d after the initiation of cherry supplementation (d 35), and 28 d after the discontinuation (d 63). Of the 89 biomarkers assessed, cherry consumption for 28 d altered concentrations of 9, did not change those of 67, and the other 13 were below the detection limits. Cherry consumption decreased ($P < 0.05$) plasma concentrations of extracellular newly identified ligand for the receptor for advanced glycation end products (29.0%), CRP (20.1%), ferritin (20.3%), plasminogen activator inhibitor-1 (19.9%), endothelin-1 (13.7%), epidermal growth factor (13.2%), and IL-18 (8.1%) and increased that of IL-1 receptor antagonist (27.9%) compared with corresponding values on study d 7. The ferritin concentration continued to decrease between d 35 and 63 and it was significantly lower on d 63 than on d 7. Because the participants in this study were healthy, no clinical pathology end points were measured. However, results from the present study demonstrate that cherry consumption selectively reduced several biomarkers associated with inflammatory diseases. J. Nutr. doi: 10.3945/jn.112.171371.

Introduction

Increased oxidative stress and inflammation are among the major causes of a number of human chronic inflammatory diseases, including type 2 diabetes mellitus (T2DM)[8], cardiovascular disease (CVD), and cancer (1–3). For example, the incidence of CVD increased 4-fold for participants in the highest quartile of C-reactive protein (CRP), a marker for inflammation, compared with those in the lowest quartile (4). Results from epidemiological studies also indicate an inverse association among fruit and vegetable intake and the risk for several chronic inflammatory diseases (5,6). Besides providing essential vitamins, minerals, and dietary fiber, fruits contain polyphenols that exhibit antioxidant, antiinflammatory, and lipid-lowering properties (7–9). Cherry powder and the bioactive functional components prepared from cherries reduced oxidative stress and inflammation in several animal models (10–14).

Results from a limited number of human studies have demonstrated the health benefits of cherries. For example, cherry consumption (227 g/d, 3 mo) relieved symptoms of arthritis in a preliminary study (15). Although we observed that consumption of a single bolus of sweet Bing cherries (280 g) following a 12-h fast by healthy women reduced the circulating concentrations of CRP and NO within 3 h of the bolus, the results did not attain significance ($P < 0.1$) (16). More recently, we demonstrated that

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[3] This trial was registered at www.clinicaltrials.gov as NCT01734070.

[4] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[8] Abbreviations used: CVD, cardiovascular disease; EN-RAGE, extracellular newly identified ligand for the receptor for advanced glycation end products; ET-1, endothelin-1; IL-1Ra, IL-1 receptor antagonist; LDD, lowest detectable dose; PAI-1, plasminogen activator inhibitor-1; T2DM, type 2 diabetes mellitus.

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the circulating concentrations of CRP and NO were significantly decreased when healthy men and women consumed sweet Bing cherries (45 cherries/d, ~280 g, 28 d) compared with the concentrations measured before the study or 28 d after discontinuation of cherries consumption (17). Other investigators reported that consumption of tart cherry juice decreased exercise-induced oxidative stress and inflammation in elderly humans as well as in marathon runners (18,19). In addition, consumption of tart cherry juice reduced exercise-induced strength loss and pain in college students (20). Thus, limited data indicate that both sweet and tart cherries decrease oxidative stress and inflammation in humans.

The results of above studies suggest that consumption of cherries may reduce the incidence of human chronic diseases. However, all previous studies were limited in scope, because each examined only a select number of response variables. With the recent advances in the gene arrays and proteomics technologies, it is now possible to examine the effects of dietary or pharmaceutical interventions on changes of global or targeted gene expression. To our knowledge, none of the other previously reported studies used such technologies in determining the effects of cherry consumption on the risk factors for chronic human diseases. The purpose of our study was to examine the effects of cherry consumption on concentrations of risk factors for multiple chronic diseases in plasma samples collected from human participants with modestly elevated CRP by using a targeted proteomic approach.

Participants and Methods

Subjects and study design. The Human Subjects Review Committee of the University of California, Davis, approved the study. Eighteen participants (2 men, 16 women) 45–61 y of age (mean ± SEM = 50 ± 1) with a BMI range of 20–30 kg/m^2 (mean ± SEM = 26.3 ± 0.9) completed a 63-d study. The study comprised 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 36–63). The details regarding participant selection and study design were previously reported (17). We planned to use individuals with elevated CRP (3–25 mg/L); however, because of the short season for fresh California Bing cherries (June to August), we relaxed this criterion and included participants with CRP values of 1–14 mg/L (mean 3.5 mg/L). The normal concentration of CRP is <1.0 mg/L (4). They were advised to not 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. Volunteers were interviewed in person on d 1, 7, 21, 35, and 63. They were instructed and reminded to limit all cherries from the diet (except those provided by the study) and limit berries to <1/2 cup/d (54 g), tea to <16 fl oz./d (474 mL), grape juice to <8 fl oz./d (237 mL), and red wine to <10 fl oz./d (296 mL). Cherries were dispensed on d 7, 14, 21, and 28 and staff members were in phone and email contact with volunteers to reinforce the dietary instructions. Notes were written for any adverse effects and when volunteers missed consuming their daily allotment of cherries or they requested additional supplies of cherries, or to change their appointments for pick up or delivery. In addition, a face-to-face interview for the 24-h dietary recall was done at d 7, 35, and 63. Twenty-four hour dietary records were analyzed by the Nutrition Data System for Research (University of Minnesota). Analysis of dietary records showed no difference in the consumption of macro- and micronutrients of interest between the 3 metabolic periods (**Table 1**). Overall, our records indicated good compliance with cherries and diets. Cherries provided ~216 kcal or 903 kJ/d, which represented ~11% of the daily energy intake. The source and storage of cherries was previously reported (17).

Sample collection. Twelve-hour fasting blood samples were drawn by venipuncture into tubes containing EDTA on study d 1 and 7 (baseline), 21 and 35 (intervention), and 63 (postintervention). We previously published some results from this cherry intervention study (17) but had additional plasma samples stored at −80°C that were analyzed for the results presented here.

TABLE 1 Estimated macro- and micronutrient intake by humans participating in the cherry study[1]

Variable	Study d 7	Study d 35	Study d 63
Energy, *kJ/d*	8120 ± 614	7740 ± 535	7750 ± 535
Protein, *energy %*	16.2 ± 0.9	14.0 ± 0.8	16.1 ± 0.7
Carbohydrate, *energy %*	50.4 ± 2.6	55.4 ± 2.6	53.0 ± 2.8
Fat, *energy %*	32.6 ± 1.9	32.0 ± 2.0	31.4 ± 2.2
SFA, *energy %*	10.5 ± 1.0	10.9 ± 0.8	11.3 ± 0.9
MUFA, *energy %*	12.3 ± 0.7	11.7 ± 1.1	11.7 ± 1.2
PUFA, *energy %*	7.1 ± 0.6	6.9 ± 0.7	5.9 ± 0.5
Total fiber, *g/d*	20.7 ± 1.8	21.9 ± 1.8	20.4 ± 1.8
Vitamin A, *IU/d*	13,000 ± 2620	12,000 ± 4350	9500 ± 1890
α-Tocopherol, *mg/d*	13.9 ± 2.5	13.6 ± 2.3	11.4 ± 2.1
Vitamin C, *mg/d*	114 ± 14.4	128 ± 30.8	154 ± 28.4
Iron, *mg/d*	20.3 ± 3.4	17.6 ± 2.6	18.4 ± 2.9
Zinc, *mg/d*	12.8 ± 2.0	11.4 ± 1.8	11.7 ± 1.4
Copper, *mg/d*	1.7 ± 0.3	1.8 ± 0.3	1.7 ± 0.2
Selenium, *μg/d*	110 ± 14.8	92.0 ± 7.9	101 ± 10.7

[1] Values are mean ± SEM, n = 18. Food intake was estimated by 24-h dietary recalls on each of the above study days. Intake of none of the nutrients listed above differed significantly during the 3 phases of the study.

Laboratory methods. Clinical and analytical methods used for the results previously reported were included in our previous paper (17).

MYRIAD RBM human MAP1.6. Because of the high cost, this analysis was performed only on plasma samples collected on study d 7 (baseline), 35 (end of cherry consumption), and 63 (28 d post cherry consumption). Samples were thawed, vortexed, and centrifuged at 13,000 × g for 5 min and 100 μL was removed for MAP analysis. Using automated pipetting, an aliquot of each sample was introduced into one of the capture microsphere multiplexes of the Human MAP 1.6. This MAP was comprised of 89 antigens that included markers for oxidative stress, inflammation, immune status, T2DM, CVD, blood clotting, and liver and kidney functions (21). All plasma samples were tested in duplicate. The samples and capture microspheres were mixed and incubated at room temperature for 1 h. Multiplexed cocktails of biotinylated and reporter antibodies were then added and incubated for an additional hour at room temperature. Multiplexes were developed with an excess of streptavidin-phycoerythrin. The volume of each multiplexed reaction was standardized by vacuum filtration or the addition of matrix buffer. Analysis was performed in a Luminex 100 instrument; the resulting data were analyzed with proprietary software developed at Rules-Based Medicine. For each multiplex, both calibrators and controls were included with each microtiter plate. The intra-sample CV for all antigens with concentrations above the lowest detectable dose (LDD) was <20%; it was <10% for >50% of the antigens tested. The inter-assay mean CVs for variables whose concentrations were altered by cherry consumption were: CRP, 7%; epidermal growth factor (EGF), 11%; extracellular newly identified ligand for the receptor for advanced glycation end products (EN-RAGE), 15%; endothelin-1 (ET-1), 5%; ferritin, 15%; IL-18, 11%; IL-1 receptor antagonist (IL-1Ra), 15%; plasminogen activator inhibitor-1 (PAI-1), 10%; and TNFα, 20%.

Statistical analysis. Thirteen of the 89 biomarkers tested had most of the readings below the lowest limit of quantification. These biomarkers and their lowest limit of quantification were: fibroblast growth factor (335 pg/mL), granulocyte-macrophage colony stimulating factor (38 pg/mL), IL-1α (0.004 μg/L), IL-1β (0.36 pg/mL), IL-2 (23 μg/L), IL-5 (4.0 pg/mL), IL-6 (1.2 pg/mL), IL-12 subunit p70 (20 pg/mL), IFNγ (2.1 pg/mL), lymphotactin (0.23 pg/mL), matrix metalloproteinase-3

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(0.13 μg/L), TNFβ (36 pg/mL), and tissue factor (0.30 μg/L). Those 13 markers were excluded from statistical analysis. Our samples did not have any biomarkers with concentrations exceeding the maximum concentration of the calibration curves. We had several samples with concentrations below the LDD; values below the LDD were assigned concentrations equal to the minimum observed value divided by 2. The SAS PROC MIXED was used to fit a repeated-measures model with a first-order autoregressive covariance structure among the repeated measures (22). Data transformation was used as appropriate to stabilize the variances among treatment periods. The fixed effect was day and the random effect was subject. One-tailed tests for single degree of freedom contrasts were used to compare the treatment periods. To determine the effects of cherries, comparisons were made between d 7 and 35, and to determine the recovery of values for the analytes to the pre- cherry consumption level, comparisons were made between d 7 and 63. Results are presented as means ± SEM. Differences were considered significant for $P < 0.05$.

Results

Dietary intake, chemical analysis, and effects of cherries on clinical panels. Dietary records collected during the 3 study periods did not show any difference in the intake of various dietary components during the study (Table 1). Polyphenol and vitamin C concentrations of the cherries and their effects on hematological, chemistry, and lipid panels were previously reported (17).

Effect of cherries on plasma concentrations of RBM MAP 1.6 analytes. Of the 89 biomarkers that comprised the MAP1.6 panel, cherry consumption decreased plasma concentrations of EN-RAGE (29.0%), ferritin (20.3%), CRP (20.1%), PAI-1 (19.9%), and EGF (13.2%), ET-1 (13.7%), TNFα (14.4%), and IL-18 (8.1%), and increased that of IL-1Ra (27.9%). All changes were significant ($P < 0.05$), except TNFα ($P = 0.07$) (**Table 2**). Concentrations of ferritin continued to decrease even after the discontinuation of cherries and was significantly different between study d 7 and 63 ($P = 0.01$). Concentrations of all other variables (CRP, PAI-1, ET-1, EN-RAGE, IL-1Ra, EGF, TNFα, and IL-18) did not significantly differ between study d 7 and 63. Consumption of cherries did not significantly alter the concentrations of 67 other analytes with values above LDD (Supplemental Table 1) and concentrations of other 13 analytes were below LDD.

Discussion

Consuming sweet Bing cherries significantly decreased circulating concentrations of CRP, EGF, ET-1, EN-RAGE, ferritin, IL-18, and PAI-1; increased IL-1Ra; and tended to decrease TNFα (Table 2). After the discontinuation of cherry consumption, the ferritin concentration significantly decreased further, whereas there were no further decreases in the concentrations of other biomarkers. The CRP concentration was maintained and that of PAI-1 minimally increased between study d 35 and 63. Those changes were most likely due to the residual effects of cherries. Concentrations of 7 of these biomarkers were completely or partially reversed after 28 d of a diet without cherries. Changes in some of these markers are consistent with reported associations in the cytokine network; e.g., CRP upregulated EN-RAGE (23) and IL-18 upregulated TNFα (24). The differences in magnitude and kinetic changes in the variables tested in response to cherry consumption and then their withdrawal may be due to the involvement of different cell types and their mechanisms of action.

The magnitude and direction of changes in CRP in our current analysis were consistent with our previous report (17) even if different analytical methods were used and samples had been stored frozen at −80°C for 7 y. Similarly, 22 of 23 common biomarkers included in the current and previous arrays were not affected by cherry consumption. Our results showing a decrease in circulating CRP after consumption of Bing cherries are consistent with those reported with tart cherry juice in marathon runners (19). However, we did not find a decrease in IL-6 as reported with tart cherry juice; circulating IL-6 in our study was below the LDD for most plasma samples. Higher plasma concentrations of IL-6 in athletes may be due to the stress of marathon racing, whereas our study participants had no such stress.

The changes in the plasma concentrations of inflammatory markers found in our study may have clinical importance for the prevention or treatment of several chronic inflammatory human diseases, including arthritis, diabetes, CVD, blood pressure, and cancer. We did not monitor clinical end points for those diseases, but the changes in the biomarkers suggest that consumption of cherries may prevent, reduce risks, or modify their severity. Decreases in the concentrations of CRP and TNFα and an increase in IL-1Ra are particularly relevant for arthritis. For

TABLE 2 Effects of sweet Bing cherries on human plasma concentrations of inflammatory and other risk factors for chronic diseases[1]

Variable[2]	Study d 7	Study d 35	Study d 63	*P* value d 7 vs. 35	*P* value d 7 vs. 63
CRP, *mg/L*	3.54 ± 0.85	2.83 ± 1.24	2.78 ± 1.01	0.02	0.15
EGF, *pg/mL*	36.4 ± 5.30	31.6 ± 8.70	34.3 ± 9.42	0.05	0.22
ET-1, *pg/mL*	25.8 ± 2.67	22.3 ± 2.61	25.5 ± 2.63	0.02	0.87
EN-RAGE, *μg/L*	0.58 ± 0.10	0.41 ± 0.08	0.59 ± 0.13	0.03	0.46
Ferritin, *μg/L*	30.7 ± 8.33	24.5 ± 8.01	22.1 ± 6.29	0.01	0.01
IL-18, *pg/mL*	240 ± 23.2	221 ± 22.2	228 ± 21.3	0.05	0.36
PAI-1, *μg/L*	20.6 ± 1.80	16.5 ± 2.16	17.6 ± 2.27	0.03	0.21
TNFα, *pg/mL*	7.44 ± 0.50	6.37 ± 0.45	7.06 ± 0.63	0.07	0.59
IL-1 receptor antagonist, *pg/mL*	49.2 ± 6.06	63.0 ± 6.96	52.7 ± 7.19	0.05	0.68

[1] Values are mean ± SEM, $n = 18$ for each variable except IL-1Ra ($n = 12$) and TNFα ($n = 6$). EN-RAGE, extracellular newly identified ligand for the receptor for advanced glycation end products; ET-1, endothelin-1; IL-1ra, IL-1 receptor antagonist; PAI-1, plasminogen activator inhibitor-1.
[2] SAS mixed-model procedures analysis.

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example, arthritic stiffness and pain was reduced in human participants by canned and fresh cherries (15). Similarly, cherry powder decreased symptoms of arthritis and plasma concentrations of IL-6 and TNFα in rats (25). This may be important, as anti-TNFα drugs are now licensed for treating certain inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease (26). In addition, IL-1Ra has been used as a therapeutic agent in the treatment of human patients with rheumatoid arthritis (27,28). Collectively, these findings suggest that increased consumption of cherries could potentially prevent or reduce the severity of arthritis.

Elevated CRP, PAI-1, ET-1, and EN-RAGE are risk factors for CVD, diabetes, and metabolic syndrome (4,29–32). PAI-1 is the major physiological inhibitor of tissue-type plasminogen activator that prevents clot formation through fibrinolysis. Plasma concentrations of PAI-1 correlate with metabolic syndrome and may predict future risk for T2DM and CVD (29). Increased expression of PAI-1 was found in atherosclerotic lesions in humans, especially atherosclerotic plaques in patients with T2DM (30). Polyphenols downregulated PAI-1 gene expression in cultured endothelial cells and also in rat aortic endothelial cells in vivo (33). Thus, our findings regarding the effects of cherries on plasma concentrations of PAI-1 are in agreement with those from studies with cultured cells and rats in vivo.

ET-1 is one of the most potent vasoconstrictors currently identified (34). It also has proliferative, profibrotic, and proinflammatory properties and may contribute to many facets of diabetic vascular disease (31). Polyphenols from grapes significantly decreased blood pressure in men with metabolic syndrome (35). However, in our study with healthy participants, there was only a trend for decreases in systolic blood pressure. Other recent research has shown that ET-1 enhances the development and growth of several types of cancer cells by affecting cell proliferation, escape from apoptosis, angiogenesis, invasion, and metastatic dissemination (36). Thus, ET-1 is a pleotropic molecule; reduction in its plasma concentrations by consuming cherries may decrease the risk for several diseases, including diabetes, hypertension, CVD, and cancer.

EN-RAGE is expressed by inflammatory cells such as mononuclear phagocytes and polymorphonuclear leukocytes of tissues. It mediates the clearance of advanced glycation products of proteins and lipids that accumulate in plasma and tissues of individuals with diabetes. Engagement of the receptor, RAGE, by the ligand, EN-RAGE, activates NFκB, a central transcription factor for inflammatory mediators and adhesion molecules (37). Plasma concentrations of EN-RAGE were twice as high in patients with diabetes compared with those without (38). The EN-RAGE concentration was positively correlated with the concentrations of CRP, hemoglobin A1C, fasting plasma glucose, and white blood cell count (38,39). Thus, a synergistic feedback loop of inflammatory mediators may be reduced when cherries are consumed, which may in turn reduce risks for diabetic complications and CVD.

IL-18 mediates the development and progression of a number of chronic inflammatory and autoimmune diseases, including arthritis, insulin-dependent diabetes, multiple sclerosis, chronic hepatitis, lupus erythematosis, psoriasis, and others (24). A decrease in the circulating concentration of IL-18 caused by cherries may reduce the risk or severity of those inflammation-related diseases.

Serum ferritin is a marker for both iron status and inflammation; its concentration is positively associated with inflammatory proteins such as CRP (40). In our study, changes in ferritin concentration were similar to those in CRP concentrations (Table 2). A positive association between ferritin concentration and other markers of inflammation has been demonstrated in several diseases (40–42). Because blood concentrations of ferritin can decrease during iron deficiency and also during a decrease in inflammation (40,43), it is possible that the decrease in the plasma ferritin concentrations observed in our study resulted from both the blood drawn during the study and decreased inflammation.

Binding of EGF to its cognate receptor leads to autophosphorylation of receptor tyrosine kinase and subsequent activation of signal transduction pathways that regulate cell proliferation, differentiation, and survival (44). EGF gene expression and EGF protein concentrations are significantly elevated in several malignancies. A decrease in the concentration of EGF caused by cherries may decrease tumor cell proliferation and survival.

Our study had several limitations and strengths. It did not have a control group, was not blinded or randomized, and enlisted relatively healthy participants. Despite these limitations, we found significant changes in the concentrations of several biomarkers. This may be because of our study design, sensitivity and precision of analytical methods, and amount and duration of the intervention. We had a successive study design in which participants acted as their own controls for the intervention, which allowed the statistical tests to be made on a within-subject basis. This enhanced the precision of the tests. The amount of cherries (280 g/d) and the 28-d intervention with water-soluble polyphenols were adequate to demonstrate significant changes. Although different participants joined the study at different days, all blood samples were collected at similar time points and analyses for specific biomarkers were performed at the same time. The complete or partial return of some of the markers to preintervention concentrations on d 63 suggests that consumption of cherries caused those changes. Parallel changes in the concentrations of several biomarkers of inflammation also indicate that changes were caused by cherries.

In conclusion, changes in the plasma concentrations of the biomarkers in our study caused by cherries suggest a potential decrease in inflammation (CRP, ferritin, IL-18, TNFα, IL-1Ra, ET-1, EN-RAGE, and PAI-1) as well as reduced risks for arthritis (CRP, TNFα, IL-18, IL-1Ra), diabetes, CVD (CRP, ferritin, ET-1, EN-RAGE, PAI-1, IL-18), cancer (ET-1, EGF), and hypertension (ET-1). Even if those are risk factors for different diseases, they are all affected by increased oxidative stress and inflammation, which may be minimized or prevented by the polyphenols in cherries. To test the clinical relevance of our findings, future studies need to be conducted in populations having diseases with an inflammatory component.

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