CALIFORNIA CHERRY ADVISORY BOARD
WASHINGTON STATE FRUIT COMMISSION

Scientific Advisory Board

Seattle, WA
March 2, 2009

California Cherry Advisory Board & Washington State Fruit Commission
Scientific Advisory Board Meeting
Hosted by Bryant Christie Inc.
March 2, 2009
Seattle, WA

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Meeting Location:

Logan Building Conference Room
500 Union Street, Suite 928
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Tel: 206-292-6340 (BCI)

Purpose:

The purpose of the Scientific Advisory Board (SAB) is to help the cherry industry to answer the following questions:

1. What do we currently know about cherry health benefits?
2. Where are the holes in the research?
3. What research in these areas is currently taking place (on other produce)?
4. What are the latest consumer trends that might influence health research?
5. What steps should the cherry industry take to further its health research efforts?

Agenda:

10:00 AM Participants Arrive

10:15 – 10:30 Welcome, Introductions, and Purpose (Andrew and Mike)

10:30 – 12:00	Status of current cherry research (Cynthia and Darshan)
12:00 – 12:30	Working lunch
12:30 – 1:30	Strengths and Weakness of current research (All) Other research currently taking place (All)
1:30 – 1:45	Break
2:00 – 2:30	Latest consumer trends (All)
2:30 – 3:00	SAB recommendations (All)
3:00	Depart for airport

TABLE OF CONTENTS

CYNTHIA THOMSON PRESENTATION …..……………………………….………….1

LITERATURE REVIEW ………………………….………………………….…...16

SELECTED PUBLISHED RESEARCH STUDIES

MEDIA COVERAGE ……..........…………………………………………42

CONSUMPTION OF CHERRIES LOWERS PLASMA URATE IN HEALTHY WOMEN …………………………………………..48

EFFICACY OF TART CHERRY JUICE BLEND IN PREVENTING THE SYNPTOMS OF MUSCLE DAMAGE …………………..…………..52

CYCLOOXYGENASE INHIBITORY AND ANTIOXIDANT CYANIDIN GLYCOSIDES IN CHERRIES AND BERRIES ……….…………..57

COMPARISON OF ANTIOXIDANT POTENCY OF COMMONLY CONSUMED POLYPHENOL-RICH BEVERAGES IN THE UNITED STATES ….…………………………….…………..65

RESEARCH PROPOSALS

EFFECTS OF BING SWEET CHERRIES ON THE CONCENTRATIONS OF CIRCULATING AND EX VIVO PRODUCED MARKETS ASSOCIATED WITH CARDIOVASCULAR DISEASE, BLOOD CLOTTING, IMMUNE STATUS, INSULIN RESISTANCE, AND DIABETES, IN HEALTHY MEN AND WOMEN …..……..……..74

ROLE OF FRESH CHERRIES IN MODULATING BIOMARKERS OF CANCER RISK AMONG MALES AT RISK FOR PROSTATE CANCER ….…………….………………………….…………..80

Sweet Cherries and Health

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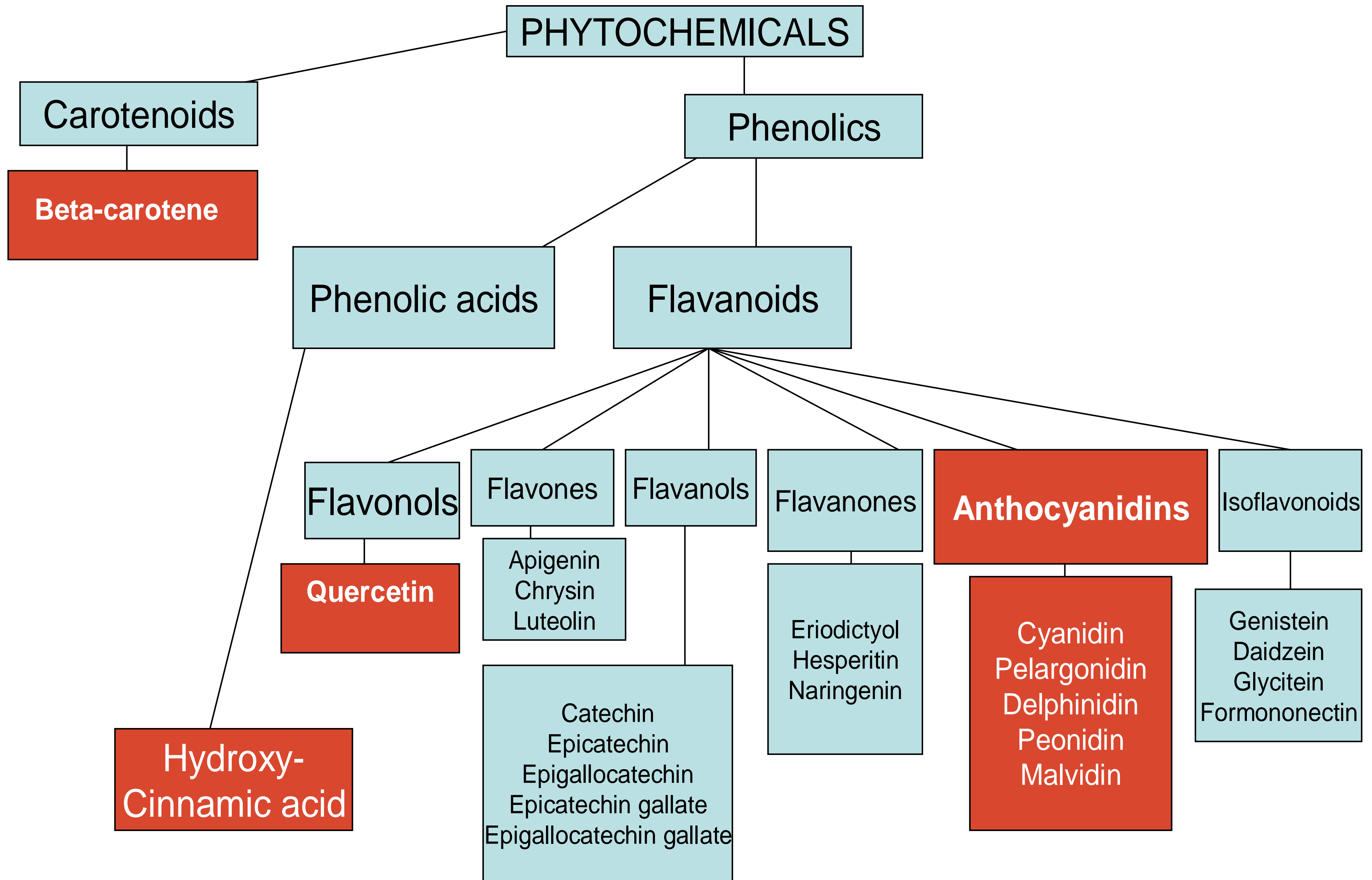
Cherries: Nutritional Highlights

- Low calorie food
- Low fat food
- Low glycemic index (compared to most fruits)
- Good source of potassium
- Good source of carotenoids
- ALSO, good source of anthocyanin and other bioactive food compounds

Nutrient, Carotenoid, Anthocyanin and Quercetin content of commonly consumed cherry products (per 100 grams or approx. 15 cherries)					
Nutrient /phytochemical	**Cherries, sweet**	**Cherries, tart**	**Cherries, sweet, canned**	**Cherries, sweet, frozen, sweetened**	**Maraschino**
Energy (kcal)[a]	**63**	50	46	89	165
Protein (g)[a]	**1.06**	1.0	0.8	1.15	0.22
Fat (g)[a]	**0.2**	0.3	0.13	0.13	0.21
Carbohydrate (g)[a]	**16.0**	12.2	11.8	22.4	42.0
Fiber (g)[a]	**2.1**	1.6	1.5	2.1	3.2
Glycemic Index[b]	**22**	22	22	22	Not available
Vitamin C (mg)[a]	**7**	10	2.2	1.0	0
Vitamin A (IU)[a]	**64**	1283	160	189	45
Potassium (mg)[a]	**222**	173	131	199	21
β-carotene (mg)[a]	**38**	770	96	113	27
Lutein/ Zeaxanthin (mg)[a]	**85**	85	57	85	59
Total anthocyanin (mg)[c]	**80.2**	6.7(?)	Not available	Not available	Not available
Quercetin (mg)[c]	**2.64**	2.92	3.2	Not available	Not available

Bioactive Food Components in Sweet Cherries



Anthocyanins in Sweet Cherries and Related Plant Foods

	Anthocyanins						Total (mg)
Plant Food (1 cup)	Cyanidin (mg)	Deophinidin (mg)	Malvidin (mg)	Pelargonidin (mg)	Peonidin (mg)	Petunidin (mg)	
Cherries, Sweet	**75.2**	0	0	0.5	4.5	0	**80.2**
Cherries, Tart	6.7	0	0	0	0	0	**6.7**
Cherries, sweet, canned	0	0	0	0	0	0	**0**
Apricots	0	0	0	0		0	**0**
Peaches	1.6	0	0	0		0	**1.6**
Plums	12.0	0	0	0		0	**12.0**
Blueberries, raw	17.0	47.4	61.4	0	11.4	26.4	**163.6**
Raspberries	35.8	0.3	0.7	1.9	0	0	**38.7**
Grapes, red	1.5	3.7	34.7	.02	2.9	2.1	**44.9**
Red Wine	0.4	1.0	7	0	0.8	0.9	**10.1**

Comparison of total anthocyanins, total phenolics, and antioxidant properties of flesh, pits, and skins of different cherry cultivars (after Chavanalikit and Wrolstad, 2004).

Cultivar	Portion	Anthocyanins (mg/100g fw)[Z]	Total phenolics (mg/ g fw)[Y]	ORAC (µmol TE/g fw)	FRAP (µmol TE/g fw)
Bing (sweet)	Flesh	26.0±0.7	1.34±0.18	9.07±0.35	7.28±0.24
	Pits	10.4±3.1	0.92±0.09	5.94±0.91	5.04±0.96
	Skins	60.6±2.5	3.33±0.41	28.26±1.10	21.05±0.55
Rainier (sweet)	Flesh	0.0±0.0	0.65±0.05	4.62±0.18	2.27±0.22
	Pits	0.1±0.0	0.54±0.04	3.38±0.26	2.00±0.13
	Skins	2.1±0.4	1.42±0.05	10.50±1.51	5.92±0.39
Montmorency (tart)	Flesh	0.0±0.09	3.01±0.29	15.00±1.00	13.81±0.26
	Pits	0.8±0.08	1.57±0.02	9.78±0.28	8.48±0.85
	Skins	36.5±1.6	5.58±0.33	51.02±1.97	47.96±1.33

Disease-specific Health Effects

Limited peer-reviewed data *specific to sweet cherries*, but disease associations speculated in relation to:

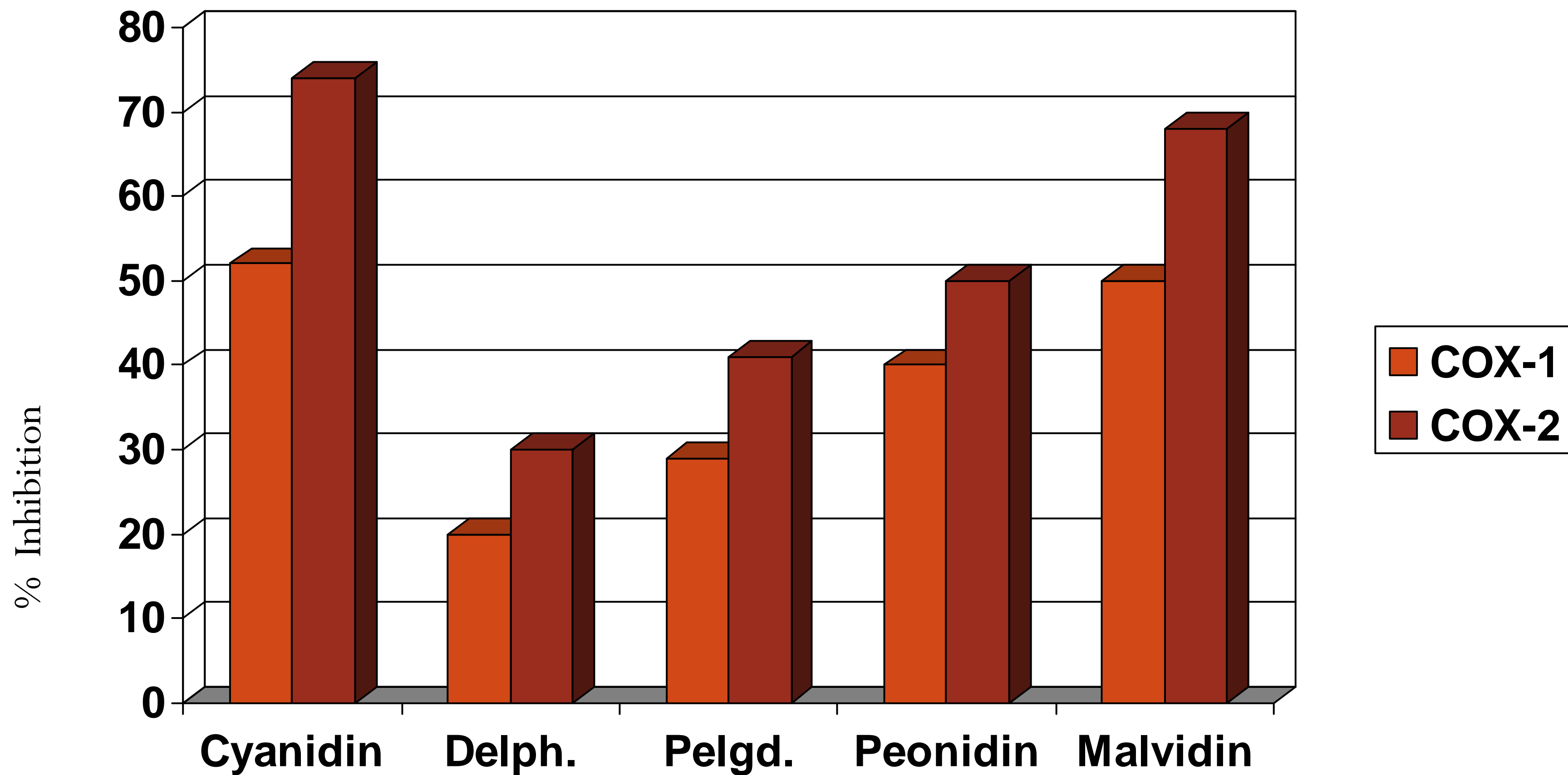
- Cancer
- Cardiovascular
- Diabetes / insulin resistance
- Arthritis
- Gout
- Alzheimers Disease

How Do BAFC in Sweet Cherries Potentially Modify Disease Risk?

- Anti-inflammatory response
- Anti-growth effects
- Differentiation-inducing agents
- Anti-oxidation / reduction in oxidative stress
- Modify insulin resistance; lower glucose levels
- Melatonin? –sleep, jetlag
- Muscle recovery post-exercise

Park EJ et al *Cancer Metas Rev*, 2002; Jayaprakasam, 2006; Tsuda, 2003

Anti-inflammatory Effects of Select Anthocyanins in Cell Culture



Individual Anthocyanins Evaluated in Cell Culture
40-mM concentrations

Adapted from: Seerman NP, Zhang Y and Nair MG, *Nutr Cancer* 2003;46(1):101-106

COX inhibition- Seeram NP, *Phytomedicine*, 2001

- HPLC showed two peak anthocyanins from cherries – anthocyanin 1 is more concentrated in tart cherries and anthocyanin 2 in sweet cherries
- Antioxidant activity (liposomal model system) slightly greater in sweet cherries
- Cox 1 inhibition: 26% for tart cherry, 29% for sweet
- Cox-2 inhibition: 38% for tart cherry, 47% for sweet cherry
- Cox inhibition proportional to anthocyanin distribution

Tart Cherry Juice and Muscle Damage

- Connolly DAJ, McHugh MP, Padilla-Zakour OI. BJSM 2006;40:679-83.
- Human Performance Lab –University of Vermont
- Sample: 14 male college students
- Randomized, placebo-controlled, cross-over design – 12 ounces tart cherry juice twice daily for 8 days; 2-week washout and cross-over
- Elbow flexion strength and self-report of elbow flexor pain significantly improved; relaxed elbow angle, muscle tenderness -NS

Current Research Needs to Advance the Health Messaging for Cherries

- Mechanistic research
- Dose-finding studies
- Human; beyond healthy volunteers
- Larger sample size
- Variety of investigators

- Dietary measurement
  - Instruments lack specificity
  - Seasonality of intake
  - Biomarkers of intake

- Collaborations: multidisciplinary, translational

- Dissemination efforts – translating science for the public

Potential Funding Sources

- USDA Bioactive Food Compounds
- Fruit and Vegetable Improvement Center for Fruit and Vegetable Research (Texas A&M)
- National Cancer Institute
  - Phase I BAFC for chemoprevention
- Industry
  - Pilot studies
  - Preliminary data for larger trials

Developing the Evidence Base for Health Messaging

- Research programming
  - Small grants
  - Research symposia
  - Peer-reviewed publications
- Scientific Advisory Board

How to Disseminate Information re: Cherries and Health

- Target Audiences
  - Dietitians, physicians, nurses, exercise physiologists
  - Health Food Stores / grocery
  - Fitness Centers / Weight Loss programs
- Mailings,
- web-ad (WebMD), Health care magazines (e.g. Arthritis Today, Prevention)
- Media Messages- manuscript-specific press releases, You-tube, FaceBook, internet marketing

SWEET CHERRIES: A RESEARCH REVIEW

Cherries like other fruits and vegetables have long been considered a healthful addition to a well-balanced diet. In 2007, the Northwest Cherry Growers commissioned a review to collect and evaluate worldwide research data on the health benefits of cherries. The study was completed by Cynthia Thomson, PhD, RD, Department of Nutritional Sciences, and Chieri Kubota, PhD, Department of Plant Sciences, at the University of Arizona.

Background: Cherries in the United States

The United States has historically been the largest exporter of cherries worldwide, followed by Turkey and Chile. U.S. cherry production has increased from 160,844 tons in 2003 to 253,286 tons in 2005, an average annual increase of 25 percent (FAO, 2007). Washington state records the highest production of sweet cherries in the U.S. (150,000 tons; USDA NASS, 2006).

The majority of sweet cherries are grown for fresh consumption, while 40 percent are processed as brined, canned, frozen, dried or used for juice. More than 50,000 tons of sweet cherries are exported annually to Canada, Japan, Taiwan, Hong Kong and other countries from the U.S.

In comparison to sweet cherries, 99 percent of tart cherries – due to their acidic flavor – are processed as frozen, canned, brined, dried, or used for juice. Processed tart cherries are primarily used in culinary service (cooking and baking). More than 10,000 tons of tart cherries are exported to Europe, Canada, Japan, Korea and other countries from the U.S. annually.

Cherry Nutrient and Phytochemical Composition

Cherries are considered a nutrient dense food, meaning that for relatively few calories, they possess a significant amount of nutrients and phytochemicals ranging from vitamin C and fiber to health-promoting bioactive food components including anthocyanins, quercetin and, to a lesser extent, carotenoids.

Nutrient, Carotenoid, Anthocyanin and Quercetin content of commonly consumed cherry products (per 100 grams or approx. 15 cherries)

Nutrient /phytochemical	Cherries, sweet	Cherries, tart	Cherries, sweet, canned	Cherries, sweet, frozen, sweetened
Energy (kcal)	63	50	46	89
Protein (g)	1.1	1.0	0.8	1.2
Fat (g)	0.2	0.3	0.1	0.1
Carbohydrate (g)	16.0	12.2	11.8	22.4
Fiber (g)	2.1	1.6	1.5	2.1
Glycemic Index	22	22	22	22
Vitamin C (mg)	7	10	2.2	1.0
Potassium (mg)	222	173	131	199
Lutein/ Zeaxanthin (µg)	85	85	57	85
Total anthocyanin (mg)	80.2	6.7	Not available	Not available
Quercetin (mg)	2.64	2.92	3.2	Not available

USDA Database for the flavonoid content of selected foods (2006). http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02.pdf

Andrew Flood, PhD; Amy F. Subar, PhD; Stephen G. Hull, MS; Thea Palmer Zimmerman, MS, RD; David J. A. Jenkins, MD, PhD, DSc; Arthur Schatzkin, MD, DrPH. Methodology for Adding Glycemic Load Values to the National Cancer Institute Diet History Questionnaire Database. J Am Diet Assoc. 2006;106:393-402.

Sweet cherries are a significant source of polyphenols in the human diet. Bing cherries contain approximately 160-170 mg total polyphenols in a 100-gram serving. The primary class of phenolics in sweet cherries is hydroxycinnamates, accounting for about 40 percent of the total.

Cherries and Anthocyanins

The following table lists the specific anthocyanins (antioxidant flavonoids) found in sweet cherries and other fruits where there is evidence suggesting demonstrated health-promoting effects related to the anthocyanin and/or polyphenol content. Sweet cherries are particularly rich in cyanidin content, constituting more than 90 percent of its total anthocyanin content.

Anthocyanins in Sweet Cherries and Related Plant Foods

Plant Food (1 cup)	Total (mg)
Cherries, Sweet	80.2
Cherries, Tart	6.7
Cherries, sweet, canned	Not available
Apricots	Not available
Peaches	1.6
Plums	12.0
Blueberries, raw	163.6
Raspberries	38.7
Grapes, red	44.9
Red Wine	10.1

USDA Anthocyanin Database, accessed March 5, 2007.
http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02.pdf

The Health Benefits of Cherries

Introduction

Diets rich in fruits and vegetables are known to reduce the risk of chronic diseases including cancer, cardiovascular disease, diabetes, obesity or select inflammatory disorders. While data on the specific health benefits of cherries is limited, in recent years the U.S. Department of Agriculture has expanded its bioactive food component database to include an analysis of anthocyanin content of select plant foods, and sweet, fresh cherries are considered to be significant sources of anthocyanins in the human diet.

Cancer

Sweet cherries have several cancer-preventive components including fiber, vitamin C, carotenoids and anthocyanins. The potential role of sweet cherries in cancer prevention lies mostly in the anthocyanin content, especially in cyanidin. Sweet cherries are a good source of cyanidins, which appear to act as an antioxidant and in this role may reduce cancer risk. In a study by Acquaviva et al, a significant increase in free radical scavenging was demonstrated with exposure to cyanidin (Acquaviva, 2003) and a separate study using human cancer cell lines demonstrated cell cycle arrest and apoptosis of mutated cells exposed to cherry anthocyanins (Lazze, 2004; Shih, 2005). Further research suggests that the growth arrest characteristics of cyanidin are likely, at least in part, to be a result of significant inhibitory effects of these cherry components on epidermal growth factor receptors (Meirers, 2001). Finally, there is compelling evidence from basic science that cyanidin may also promote cellular differentiation and thus reduce the risk for healthy cells to transform to cancer (Serafino, 2004).

Cardiovascular Disease

The role of red wine in reducing the risk of cardiovascular disease has been studied widely for more than 20 years, and studies suggest anthocyanin found in red wine has important biological effects that reduce cardiovascular disease risk (Corder, 2006). This includes protecting lipids from oxidant damage and cardiovascular vessel plaque formation, anti-inflammation, nitric oxide formation and vascular dilation. Similarly, sweet cherries have been shown to have significant levels of anthocyanins as well as other pigments in perhaps smaller concentrations that together provide synergistic effects thought to be protective to heart and related vascular tissue (Reddy, 2005).

Diabetes

Evidence suggesting a protective role for cherries for diabetes is relatively rare, but researchers are interested in the role of anthocyanins in reducing insulin resistance and glucose intolerance. In one study, cells exposed to various glucose loads and then exposed to anthocyanins and anthocyanidins showed increased insulin production, suggesting the role of these compounds in blood glucose control should be explored further (Jayaprakasam, 2005). The study suggested that the bioactive compounds found in cherries are responsive, in terms of enhanced insulin production, to a glucose-rich environment and work to control glucose levels.

Recently the role of the glycemic index in diabetes control has gained renewed interest. Sweet cherries have an estimated glycemic index of 22, generally lower than other fruits including apricots (57), grapes (46), peaches (42), blueberries (40) or plums (39). The lower glycemic index makes sweet cherries a potentially better fruit-based snack food (as compared with many other fruits) for people with diabetes. The lower glycemic response shown in relation to cherry consumption may be the result of glucose-lowering effects of cherry phytochemicals in combination with the relatively modest fiber content of cherries.

Inflammation

An important new area for nutrition research is the role of naturally occurring compounds, primarily in plant foods, to modify the inflammatory process in humans. Low-grade inflammation is a potential risk factor for a wide range of chronic illnesses including cancer, cardiovascular disease, and arthritis. In addition, obesity has been shown to be associated with elevated inflammatory response. While Americans are often advised to take low-dose aspirin to offset this problem, researchers are looking for new ways – such as diet modification – to enhance anti-inflammatory response.

Select phytochemicals in cherries have been shown to inhibit the cyclooxygenase (COX) enzymes responsible for inflammatory response. In a cell culture study assessing COX-1 and -2 enzyme activity, the anthocyanin cyanindin, common to sweet cherries, along with malvidin, were shown to have the greatest inhibitory effects (Seernam, 2003). In relation to anti-inflammatory properties, cherries have been investigated in relation to pain control. Evidence suggesting a role of dietary constituents in reducing pain is limited, but remains an active area of research. (Tall, 2004).

Alzheimer's Disease

Flavonoids and procyanidin compounds have been shown to reduce oxidant stress and -amyloid production and may indirectly reduce the risk for Alzheimer's disease (Yoshimura, 2003; Heo, 2004). Recent studies have shown the potential role of sweet cherry phenolic compounds in protecting neuronal cells involved in neurological function. The phenolics in sweet cherries include both quercetin and hydroxycinnamic acid as well as anthocyanins. One study exposed neuronal cells to a variety of phenolic compounds found in sweet and tart cherries and showed that total phenolics, and predominantly anthocyanins, demonstrated a dose-dependent reduction in oxidant stress (Kim, 2005). Further study into possible protective effects of sweet cherry bioactive compounds in reducing risk for, or morbidity related to, Alzheimer's disease is warranted.

Other Potential Health Promoting Nutrients and Phytochemicals

Potassium

Sweet cherries are considered a good source of dietary potassium, with approximately 260 mg potassium for every cup of fresh cherries consumed (USDA MyPyramid nutrient data analysis program). In the past ten years, there has been increasing evidence of the importance of adequate potassium intake in reducing the risk for hypertension and stroke risk as well as other causes of morbidity (He, 2003). More than half of all American adults have high blood pressure levels. A diet high in potassium and calcium, and low in sodium and alcohol, is a reasonable and safe approach to promote blood pressure control.

Quercetin

Sweet cherries also contain a small amount of quercetin (Dunnick, 1992). Quercetin is among the most potent in terms of antioxidant activity. The ability of quercetin to act as a free radical scavenger suggests it could play a beneficial role in reducing reactive oxygen species (ROS) (i.e. hydrogen peroxide, superoxide anion) associated with chronic diseases such as cardiovascular disease and cancer (Johnson, 2000; Wilms, 2005).

Melatonin

Melatonin is a hormone produced by the pineal gland that in addition to antioxidant activity also plays a role in promoting healthy circadian rhythm and thus promoting healthy sleep patterns. Cherries are one plant food source of melatonin and melatonin levels have been estimated to be higher in tart cherries as compared to sweet cherries. In one study, melatonin supplementation appears to be effective in reducing jet lag (Herxheimer, 2002; Suhner, 2001). In combination with other behavioral approaches to promote sleep or reduce jet lag, sweet cherry intake in usual amounts could prove to be useful. Again, more research is needed.

Conclusion

While there is more room for study on the role of cherries in a healthy diet, the available research suggests that cherries – and especially the presence of antioxidant flavonoid anthocyanin in cherries – can play a role in reducing the risk of diseases including cardiovascular disease, diabetes, obesity and select auto-immune disorders when consumed as part of an overall plant-rich, healthy diet.

For More Information

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Cherries and Health: A Review

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Cherries and in particular sweet cherries are a nutrient dense food containing bioactive food components. The U.S. produced over 253 thousand tons of sweet cherries in 2005 with the majority (60%) for the consumption of fresh fruit and the rest processed. UV concentration, degree of ripeness, post harvest storage conditions, and processing each can significantly alter amounts of bioactive components. In cherries these include amounts of anthocyanins, quercetin, hydroxycinnamates, potassium, fiber, vitamin C, carotenoids and melatonin that suggest health benefits related to cancer, cardiovascular disease, diabetes, inflammatory disease (such as gout and arthritis) and Alzheimer's disease. Cherries exhibit relatively high antioxidant activity and low glycemic index. Content of cyanidin and mylvelin have been shown to inhibit COX 1 and 2 enzyme activities and other anticarcinogenic effects in in vitro *and animal experiments The current mechanistic research findings should be further substantiated through the implementation of well designed human cherry feeding studies.*

Keywords: cherry, anthocyanin, antioxidant, cancer, diabetes, harvest, health

INTRODUCTION

Cherry is a fruit belonging to the genus *Prunus* in the Rosaceae family, which contains over several hundred species distributed across northern temperate regions. The sweet cherry (*P. avium*) is native to Europe and western Asia with the most common cultivars grown in the U.S. being Bing, which produces large black firm fruits, while the tart cherry (*P. cerasus*) is produced from the Montmorency cultivar. The cherry fruit is considered a nutrient dense food with relatively low caloric intake and a significant amount of quality nutrients and phytochemicals. These range from vitamin C and fiber to select health-promoting bioactive food components including anthocyanins, quercetin and, carotenoids. Research has demonstrated several relevant biological activities that are enhanced or inhibited by constitutive components of sweet cherries and thus hold potential for reducing cancer, cardiovascular disease, diabetes and other inflammatory diseases. The primary biological mechanisms of interest include research assessing reductions in oxidant stress, inflammation and/or tumor suppression, glucose control, and inhibition of uric acid production. This review provides information on the nutrient and bioactive food components in cherries, mechanism of action, bioactivity and associated disease risk reduction.

NUTRIENT AND BIOACTIVE FOOD COMPONENTS

Data regarding the nutrient and phytochemical content of cherries and cherry products consumed in the U.S. (Table 1) and the nutritional composition of cherries in comparison to other *Prunus* genus fruits (Table 2) illustrate that sweet cherries are comparatively a good source of fiber, potassium and anthocyanins.

Table 1. Nutrient, Carotenoid, Anthocyanin and Quercetin content of commonly consumed cherry products (per 100 grams or approx. 15 cherries)					
Nutrient /phytochemical	**Cherries, sweet**	**Cherries, tart**	**Cherries, sweet, canned**	**Cherries, sweet, frozen, sweetened**	**Maraschino**
Energy (kcal)[a]	63	50	46	89	165
Protein (g)[a]	1.06	1.0	0.8	1.15	0.22
Fat (g)[a]	0.2	0.3	0.13	0.13	0.21
Carbohydrate (g)[a]	16.0	12.2	11.8	22.4	42.0
Fiber (g)[a]	2.1	1.6	1.5	2.1	3.2
Glycemic Index[b]	22	22	22	22	Not available
Vitamin C (mg)[a]	7	10	2.2	1.0	0
Vitamin A (IU)[a]	64	1283	160	189	45
Potassium (mg)[a]	222	173	131	199	21
β-carotene (μg)[a]	38	770	96	113	27
Lutein/ Zeaxanthin (μg)[a]	85	85	57	85	59
Total anthocyanin (mg)[c]	80.2	Not available	Not available	Not available	Not available
Quercetin (mg)[c]	2.64	2.92	3.2	Not available	Not available

[a] USDA National nutrient database for Standard Reference, Version 19 (2006).
[b] Glycemic Index database based on CSFII 96 data, National Cancer Institute (2004)
[c] USDA Database for the Flavonoid Content of Selected Foods, Release 2.1 (2007)

Potassium

Sweet cherries are considered a good source of dietary potassium with approximately 260 mg potassium for every cup of fresh cherries consumed (USDA MyPyramid nutrient data analysis program). In the past decade there has been increasing evidence of the importance of adequate potassium intake in reducing the risk for hypertension and stroke risk as well as other causes of morbidity (He, 2003). Over half of all American adults have high blood pressure levels, thus promoting diets high in potassium and calcium, as well as reduced in sodium and alcohol, is a reasonable and safe approach to promote blood pressure control.

Table 2. Nutrient composition of fruits within the genus *Prunus* (values per 100 grams or approximately 15 cherries)[a].

Nutrients	Sweet cherry	Tart cherry	Japanese sweet cherry[b]	Apricot	Plum	Peach
Energy (kcal)	63	50	60	48	46	39
Fiber (g)	2.1	1.6	1.2	2.0	1.4	1.5
Total sugars (g)	12.82	8.49	unk	9.24	9.92	8.39
Sucrose (g)	0.15	0.8	unk	5.87	1.57	4.76
Glucose (g)	6.59	4.18	unk	2.37	5.07	1.95
Fructose (g)	5.37	3.51	unk	0.94	3.07	1.53
Vitamin A (IU)	64	1283	163.33	1926	345	326
Vitamin C (mg)	7	10	10	10	9.5	6.6
Vitamin E (mg)	0.07	0.07	0.5	0.89	0.26	0.73
Potassium (mg)	222	173	unk	259	157	190
β carotene (μg)	38	770	unk	1094	190	162
Anthocyanins (mg)	80.19[d]	Not available	0.5[c]	Not available	12.02[d]	1.61[d]

Several mechanisms have been proposed and evaluated in relation to the reduction in blood pressure and stroke risk associated with potassium intake. Of particular importance is the concurrent lowering of sodium intake which is more easily achieved with the integration of high potassium fruits since most fruits, including cherries, are free of sodium. The shift from high sodium/low potassium to low sodium / higher potassium has been suggested to promote diuresis, reduce sympathetic nervous activity that leads indirectly to stimulation of angiotensin II and norepinephrine (Vaskonen, 2003).

A 2001 report in the American Journal of Hypertension, suggested that Americans consume additional potassium-rich foods to achieve an intake of 4700 mg/day, well above the estimated usual intake of 1740 mg/day among participants enrolling in the DASH dietary intervention trial (Appel, 1997). And the DASH trial supported the efficacy of such an approach (Sacks, 2001) although very high adherence may be essential to long term protective effects (Folsom, 2007). However, it is important to understand that an increase in *dietary* potassium intake alone, even in combination with sodium restriction generally is not associated with a significant improvement in blood pressure control (Davis, 1994) but a combination of higher potassium, higher calcium, lower sodium intake and weight control is efficacious in reducing blood pressure in people with hypertension (Wexler, 2006; Elmer,

2006). A recent meta-analysis suggests that these same dietary approaches are associated with a significant reduction in stroke risk (Ding, 2006).

Figure 1 illustrates the relationship among select bioactive phytochemical compounds in terms of chemical classification. Sweet cherries are a significant source of polyphenols in the human diet. Bing cherries contain an estimated 160-170 mg total polyphenols in a 100 gram serving. Table 3 provides currently available data regarding the antioxidant capacity of select cherry cultivars. The antioxidant activity associated with sweet cherry intake is largely related to the individual and synergistic antioxidant effects of nutrients such as vitamin C and bioactive food components in sweet cherries such as anthocyanins, quercetin, etc.

Anthocyanins

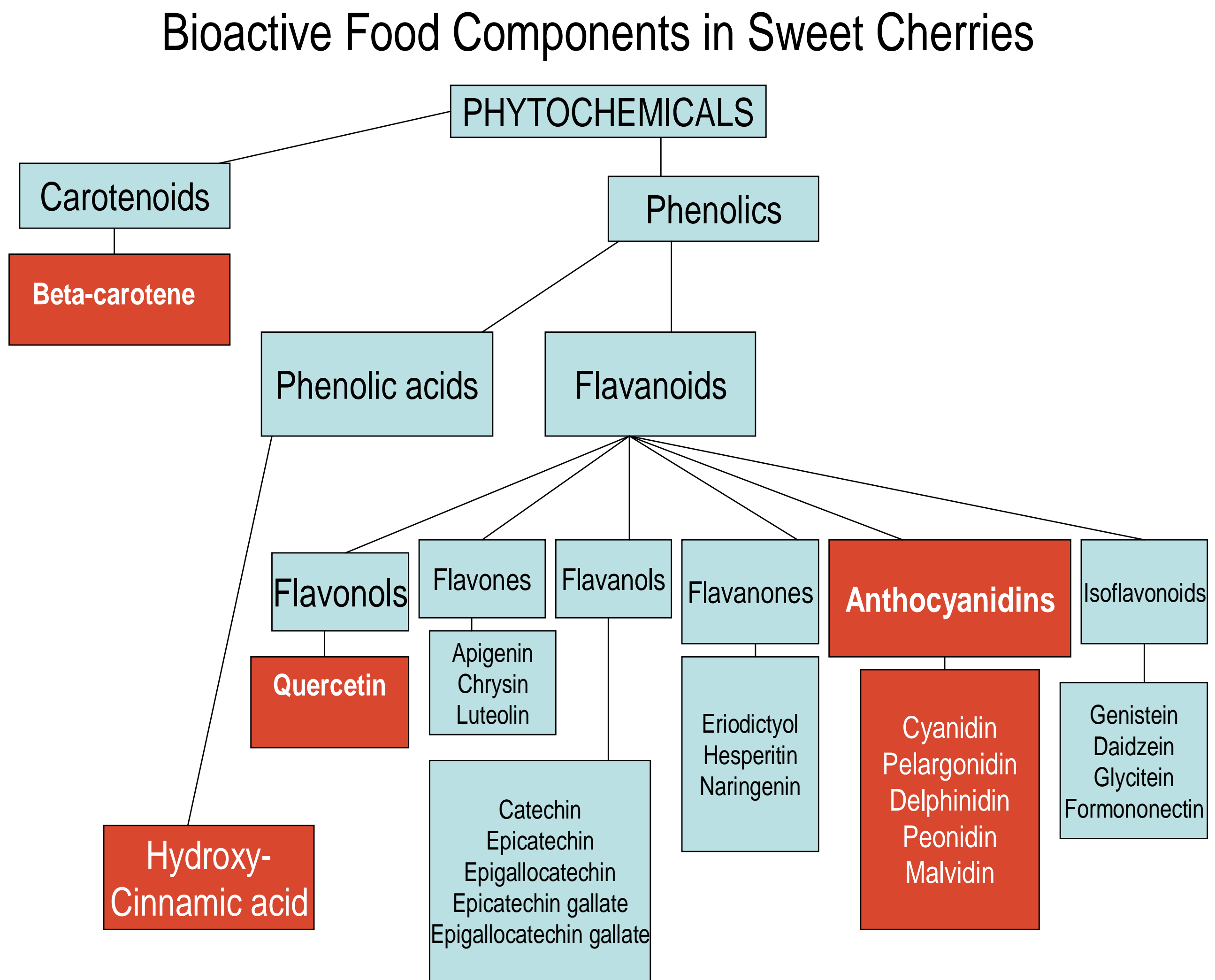
Both sweet cherries and tart cherries contain substantial amounts of anthocyanins and polyphenolics (e.g., Gao and Mazza, 1995), yet comparative data on sweet and tart cherry composition using the same analytical methodologies are limited (Chaovanalikit and Wrolstad, 2004). Table 3 describes the anthocyanin, phenolic and antioxidant content of select cherry cultivars. Bing sweet cherries were highest in anthocyanins, whereas Montmorency tart cherries were highest in total phenolics and antioxidant activities (Chaovanalikit and Wrolstad, 2004). Anthocyanin deposition in Bing sweet cherries is in the skins and flesh, while deposition in Montmorency tart cherries is limited to the skins. Seeram et al. (2002) reported that sweet cherries had the highest antioxidant activity followed by blueberries, and have a greater anti-inflammatory activities then Montmorency tart cherries. In contrast, ORAC and FRAP analyses showed that the edible portion of Montmorency tart cherries showed a greater antioxidant activity than those in sweet cherries (Chaovanalikit and Wrolstad, 2004). Considering issues of inconsistency regarding antioxidant activity measurements, the comparison of antioxidant activities between sweet cherries and tart cherries should be considered as inconclusive and requiring further investigation.

Table 3. Comparison of total anthocyanins, total phenolics, and antioxidant properties of flesh, pits, and skins of different cherry cultivars (after Chavanalikit and Wrolstad, 2004).

Cultivar	**Portion**	**Anthocyanins (mg/100g fw)**^{Z}	**Total phenolics (mg/ g fw)**^{Y}	**ORAC (µmol TE/g fw)**	**FRAP (µmol TE/g fw)**
Bing (sweet)	Flesh	26.0±0.7	1.34±0.18	9.07±0.35	7.28±0.24
	Pits	10.4±3.1	0.92±0.09	5.94±0.91	5.04±0.96
	Skins	60.6±2.5	3.33±0.41	28.26±1.10	21.05±0.55
Rainier (sweet)	Flesh	0.0±0.0	0.65±0.05	4.62±0.18	2.27±0.22
	Pits	0.1±0.0	0.54±0.04	3.38±0.26	2.00±0.13
	Skins	2.1±0.4	1.42±0.05	10.50±1.51	5.92±0.39
Montmorency (tart)	Flesh	0.0±0.09	3.01±0.29	15.00±1.00	13.81±0.26
	Pits	0.8±0.08	1.57±0.02	9.78±0.28	8.48±0.85
	Skins	36.5±1.6	5.58±0.33	51.02±1.97	47.96±1.33

^{Z} cyn-3-glu equivalent
^{Y} gallic acid equivalent
USDA Database for the Flavonoid Content of Selected Foods, Release 2.1 (2007)

Fig. 1 Bioactive Food Components in Sweet Cherries



Source: Liu RH, *J Nutr* 2004;134(suppl 12):3480S

Table 4 lists the specific anthocyanins found in sweet cherries as well as other commodity fruits. The anthocyanin content of cherries is compared to other plant foods for which evidence has suggested health promoting effects related to anthocyanin and/or polyphenol content (REFS). It is noteworthy that sweet cherries are particularly rich in cyanidin content, constituting over 90% of its total anthocyanin content. The primary distinction between sweet and tart cherries in terms of phytochemical content is the greater concentration of anthocyanins in sweet cherries.

Quercetin

Sweet cherries contain quercetin, a phenolic phytochemical belonging to a class of bioflavonoids that are widely distributed in a plant-based diet (Dunnick, 1992). Quercetin is among the most potent in terms of antioxidant activity. The ability of quercetin to act as a free radical scavenger suggests it could play a beneficial role in reducing reactive oxygen species (ROS) (i.e. hydrogen peroxide, superoxide anion) associated with chronic diseases such as cardiovascular disease and cancer (Johnson, 2000; Wilms, 2005). The unique catechol structure of quercetin, which possesses two hydroxyl groups at neighboring positions, allows for a greater level of radical scavenging activity as compared with most antioxidants (Murota, 2003). High doses of quercetin (10-100uM) have been shown to diminish malondialdehyde concentration (Alia, 2005 epub), and *in vitro* pre-treatment of

Table 4. Anthocyanins in Sweet Cherries and Related Plant Foods

	Anthocyanins						**Total (mg)**
Plant Food (1 cup)	Cyanidin (mg)	Deophinidin (mg)	Malvidin (mg)	Pelargonidin (mg)	Peonidin (mg)	Petunidin (mg)	
Cherries, Sweet	75.2	0	0	0.5	4.5	0	80.2
Cherries, Tart	6.6	Not available	Not available	Not available	Not available	Not available	Not available
Peaches	1.6	0	0	0	0	0	1.6
Plums	12.0	0	0	0	0	0	12.0
Blueberries, raw	17.0	47.4	61.4	0	11.4	26.4	163.6
Raspberries	35.8	0.3	0.7	1.9	0	0	38.7
Grapes, red	1.5	3.7	34.7	.02	2.9	2.1	44.9
Red Wine	0.4	1.0	7	Not available	0.8	0.9	Not available

human lymphocytes with quercetin (low concentrations 1-10uM quercetin) is very effective in preventing induced oxidative DNA damage in a concentration-dependent manner (Wilms, 2005).

In relation to cardiovascular disease risk reduction both oxidative stress and antiplatelet effects of quercetin have been evaluated. Human studies focused on quercetin feeding have shown mixed results on oxidative stress levels both supporting (Boyle, 2003; McAnlis, 1999; Lean, 1999) and not supporting (Beatty, 2000) a statistically relevant effect. A supplementation trial conducted among 27 healthy adults showed no significant improvement if platelet aggregation or lipid levels after consuming 1 gram/day for 28 days (Conquer, 1998); however, oxidant stress biomarkers were not assessed and selection of a healthy population may have limited the opportunity to modify existing biomarker levels. Further, concentrations of quercetin used in *in vitro* studies which support anti-platelet effects are likely not plausible in human feeding studies (Janssen, 1998). In a comprehensive review by Prior (2003) the bioavailability and antioxidant capacity of quercetin *in vivo* (as compared to *in vitro*) was reduced in relation to conjugation with glucuronide or sulfate and short half-life. Therefore, while significant antioxidant effects can occur and have been demonstrated in humans, sufficient and repeated "dosing" may be necessary in order to achieve sustained biological affects.

Studies investigating the modulation of inflammatory vascular biomarkers in relation to quercetin specifically are limited. In rat *in vivo* studies, quercetin was shown to have vasorelaxant effects (Woodman, 2004). In addition, a review of flavonoid effects on cyclooxygenase-2 supports strong inhibitory effects (O'Leary, 2004). One of the more promising studies on the health-promoting effects of quercetin in humans was a 1999 trial in which 30 males with prostatitis were randomly assigned to placebo or 500 mg quercetin twice daily for 30 days (Shoskes, 1999). Results showed a significant reduction in NIH chronic prostatitis symptom score in those randomized to quercetin (P = 0.003). No follow up trials have been conducted to further support this initial research. This daily dose is well above what could be achieved via the promotion of sweet cherry consumption.

It is important to note that the quercetin content alone in an average serving of cherries is insufficient to expect any significant effect on oxidant stress or inflammatory biomarkers, but in conjunction with other antioxidant and anti-inflammatory phytochemicals modulation of this biomarkers may be observed. Further, while sweet cherries are available source of quercetin in the human diet, citrus fruits and onions, among other fruits and vegetables, are considerable higher in quercetin content. Well controlled feeding studies are needed to assess more clearly the role of sweet cherries in modifying oxidant stress and inflammation, perhaps even a study designed to compare the modulation of these biomarkers in relation to cherry intake versus isolated quercetin.

Hydroxycinnamate

The primary class of phenolics in sweet cherries is hydroxycinnamates, comprising approximately 40% of the total (REF). Significant evidence for the role of phenolics in health has been published (REF), yet limited data regarding the health promoting effects specific to hydroxycinnamates is available. Analytical assays have been developed to qualify hydroxycinnamate levels in human urine samples (Nielsen, 2003; Bourne, 1998) and plasma (Cremin, 2001), suggesting that evaluating the relevance of these compounds in terms of health-promoting potential of cherry intake is possible.

SELECT HEALTH BENEFITS

Cancer

Sweet cherries have several cancer-preventive components including fiber, vitamin C, carotenoids and anthocyanins. The role of sweet cherries in cancer prevention lies predominantly in the anthocyanin content. While cherries are a fair source of dietary fiber and dietary fiber has been associated with reduced risk for select cancers including colorectal cancer, this association remains inconclusive (Rock, 2007). Also, the amount of fiber in a single serving of sweet cherries (2.1 grams/15 cherries) would be insufficient to modify risk if cherries were the sole source of fiber in the diet. However, certainly the added fiber associated with fresh sweet cherry intake contributes to the possibly cancer-preventive recommended dietary intake level of 30 or more grams daily (ACS, 2006). Cherries also provide a reasonable source of lutein and beta-carotene in the diet, although not near the levels associated with consumption of green leafy vegetables and orange-yellow vegetables such as carrots and sweet potatoes. Again, the presence of beta-carotene likely contributes to the total antioxidant effects of cherries, but not to any significant degree.

Of primary interest in terms of health promotion are the anthocyanins and in particular the anthocyanin cyanidin. Sweet cherries are a good source of cyanidin and the presence of cyanidin appears to have particular importance in terms of reducing cancer risk. Anthocyanins are responsible for the red-purple color inherent to fresh sweet cherries. Anthocyanin concentration is one factor that differentiates the sweet cherry from the tart cherry in that while both contain anthocyanins, sweet cherry concentrations are more than 10-fold higher, particularly in relation to cyanidin content (USDA,

2006). Thus, while literature exists suggesting that the anthocyanin content of tart cherries is health promoting, in all likelihood this evidence would be even stronger for the sweet, dark red cherry varieties given the higher anthocyanin content.

Using a mouse model of colorectal cancer, a multiple regime feeding trial was conducted. Mice were fed one of the following: 1) a cherry diet, 2) anthocyanins, 3) cyaniding, 4) control diet or 5) control diet with added sulidac (anti-inflammatory) to determine the effects of these diets on tumor development (Kang, 2003). Results suggested that mice assigned to any of the three test diets showed significantly fewer and smaller volume cecal tumors, but not colonic tumors, than control or sulindac supplemented mice. These data suggest that the bioactivity of cyanidin is responsible for inhibiting cecal tumors, but this anti-tumor effect is specific to the cecal and not colonic tumors. Similar cancer-protective effects of cyaniding glucosides have been demonstrated in studies employing cancer cell lines (Chen, 2005) including apoptotic effects via G2/M growth cycle arrest.

Further, cyanidin has also been shown to act as a potent antioxidant in research employing cell culture models. In a study by Acquaviva et al. a significant increase in free radical scavenging was demonstrated with exposure to cyanidin (Acquaviva, 2003) and a separate study using cancer cell lines from humans also demonstrated cell cycle arrest and apoptosis of mutated cells exposed to cherry anthocyanins (Lazze, 2004; Shih, 2005). Further research suggests that the growth arrest characteristics of cyanidin are likely, at least in part, to be a result of significant inhibitory effects of these cherry components on epidermal growth factor receptor (Meirers, 2001). Finally, there is compelling evidence that cyanidin may also promote cellular differentiation and thus reduce the risk for transformation of ephithelial cells to cancer (Serafino, 2004).

Wang showed potent inhibition of tumor necrosis factor alpha in relation to quercetin treatment, a bioflavonoid found in sweet cherries, and at the same time this same compound was induced by anthocyanins (also found in sweet cherries), suggesting counter-regulatory effects on cancer growth may be associated with sweet cherry consumption (Wang, 2002).

To date, no human intervention trials assessing the role of cherries and / or cherry bioactive food compounds have been completed to assess the efficacy of a cherry-enriched diet or cherry phytochemical enriched diet on cancer outcomes. In addition, while available epidemiological data suggest fruits are protective against select cancers, no data specific to cherry intake patterns are available to test hypotheses specific to cherry intake and cancer risk. Based on the ever-expanding mechanistic research from cell culture and animal models, human cherry feeding trials should be pursued to test efficacy of cherries and cherry bioactive constituents in modulating intermediate biomarkers of cancer risk.

Cardiovascular disease

The role of red wine in cardiovascular disease risk reduction has been investigated broadly for over two decades and suggests that the content of anthocyanin from red wine exerts important biological effects that reduce cardiovascular disease risk (Corder, 2006). These effects or activities include protecting lipids from oxidant damage and ensuing cardiovascular vessel plaque formation, anti-inflammation, nitric oxide formation and vascular dilation. Similarly, sweet cherries have been shown to have significant levels of anthocyanins as well as other pigments in perhaps smaller concentrations that together provide synergistic effects thought to be protective to heart and related vascular tissue (Reddy, 2005).

As with anti-cancer effects, much of the research suggesting cardio-protective effects of cherry constituents lies in well-designed cell culture models. In one study endothelial cells were removed from bovine arteries and exposed to cyanidin-3-glycoside for several hours. This treatment was associated with a significant increase in nitric oxide output and thus could be associated with a significant reduction in local oxidant stress to the cardiac tissue (Xu, 2004). Both plaque formation and

blood pressure control would be expected to result from this biological activity of cyanidin-3-glycoside. In a study using tart cherry seed extract, rat hearts were subjected to ischemic injury (which generally results in irregular and rapid heart beats and possibly heart attack) and exposed to the cherry extract at variable doses. Extract at moderate doses was associated with reduced incidence of irregular and rapid heart rates as well significantly less cardiac damage as a result of heart attacks that did occur (Bak, 2006). Repetition of this model system to test cardioprotective effects specific to sweet cherries is indicated; similar effects would be expected.

Expanding on this evidence, in 2002 Frank and colleagues investigated the role of the anthocyanin, cyanidin-3-O-glucoside, common to the cherry fruit in reducing lipid levels in rats. While anthocyanin supplementation in the diet (in this study derived from blackcurrant and elderberry and not specifically sweet cherry) did not reduce serum cholesterol levels, it did modify vitamin E levels in vital organs, suggesting an overall and indirect antioxidant effect (Frank, 2002). In another animal study, investigators targeted the cholesterol transport pathways in assessing the role of anthocyanins in reducing cardiovascular disease risk. The study isolated foam cells, key players in plaque formation within vessel walls, from mice and then exposed them to variable doses of cyanidin-3-O-β-glucoside. Results suggested that there was a dose-dependent removal of cholesterol from macrophages and their associated foam cells, illustrating a protective effect of this anthocyanin in reducing cardiovascular risk (Xia, 2005).

Clearly, this preliminary evidence suggests that the role of cherries and cherry bioactive components in protection against cardiovascular disease is an area ripe for focused research. Given the expected tolerance and acceptance of cherries in human populations, human feeding trials assessing effects of cherry intake on heart health are an important next step toward advancing our understanding. Feeding studies would also provide important information as to the appropriate “dose” of cherries to optimize cardiovascular risk reduction, should evidence from basic and animal models translate to human cardiovascular disease.

Diabetes

Evidence suggesting a protective role of cherries and cherry components in the setting of diabetes is relatively sparse. Yet, mechanistic studies in cancer and cardiovascular disease targeting common biological pathways for disease promotion, including both antioxidant and anti-inflammatory effects of cherries/cherry components point to diabetes as another potential disease target to assess the health-promoting effects of cherries.

Current evidence lies in the role of anthocyanins in reducing insulin resistance and glucose intolerance. In a cell culture study, anthocyanins and anthocyanidins in cherry fruit were combined with various glucose loads to result in a significant insulin production by anthocyanin and anthocyanidin-enriched cells (Jayaprakasam, 2005). This suggests that these bioactive compounds found in cherry fruit are responsive, in terms of enhanced insulin production, to a glucose-rich environment and work to control glucose levels. Reportedly traditional Chinese literature has illustrated the use of cherry fruit to control blood glucose for centuries (Yamahara, 1981).

In a few studies using mouse models of hyperglycemia, similar glucose-lowering effects were demonstrated in relation to feeding of either cherry anthocyanins (Jayaprakasam, 2006) or 3-o-β-d-glucoside specifically (Tsuda, 2003). In both studies, high fat diets were used to induce obesity and hyperglycemia and then supplemental feedings of cherry-specific bioactive components were provided. Protective effects were shown including reduced triglyceride synthesis as well as reduced glucose and leptin levels. Recent work by Seymour et al. (2007) found rat diets enriched with tart cherries significantly reduced levels of triglyceride, total cholesterol, insulin and markers of oxidative stress.

Recently the role of glycemic index in diabetes control has gained renewed interest. Sweet cherries have an estimated glycemic index of 22, generally lower than other fruits such as apricot (57), grapes (46), peach (42), blueberry (40) or plum (39) (Foster-Powell, et al. 2002). The lower glycemic index makes sweet cherries a potentially more appropriate fruit-based snack food (as compared with many other fruits) for people with diabetes. The lower glycemic response shown in relation to cherry consumption may be the result of glucose-lowering effects of cherry phytochemicals in combination with the fiber content of cherries.

Inflammation

An important new area for nutrition research is the role of naturally-occurring compounds in the food supply (primarily plant foods) to modify the inflammatory process in humans. It has been well recognized that low grade inflammation is a potential risk factor for a wide range of chronic illnesses including cancer, cardiovascular disease, and arthritis. Further, obesity itself has been shown to be associated with elevated inflammatory response. To reduce inflammation many Americans with or at risk for chronic inflammatory related illnesses are adviced to take low-dose aspirin or non-steroidal anti-inflammatory medications. However, these medication-based approaches are not without undesirable side-effects and thus more tolerable approaches, such as dietary modification to enhance anti-inflammatory response, are warranted.

Cherries, and the constitutive phytochemicals, have been demonstrated to inhibit the cyclooxygenase (COX) enzymes responsible for inflammatory response. In a cell culture study assessing COX-1 and -2 enzyme activity, the anthocyanin cyanindin, common to sweet cherries, along with malvidin, were shown to have the greatest inhibitory effects (Seernam, 2003). The research also indicated that cyanidin had greater anti-inflammatory activity via COX enzyme inhibition than polyphenols found in green tea. The strong inhibitory potential of cyanidin is thought to be the result of the chemical structure which exhibits a hydroxyl group positioned in the B ring of the compound. These data provide evidence of anti-inflammatory effects that should be investigated in human feeding studies using fresh cherries as the dietary intervention and examining COX 2 activity as well as select inflammatory biomarker outcomes.

These results are further substantiated by another cell-culture study comparing the anti-inflammatory effects of cyanidin alone, anthocyanins from a wide variety of cherries and common anti-inflammatory medications (Seeram, 2001). The results of this study show that sweet cherries (Montmorency) inhibited COX-1 enzyme activity by an average 30% and COX-2 activity by 48%. This inhibitory response on inflammatory enzyme activity was approximately 60% of the Cox-1 inhibition demonstrated for the anti-inflammatory medications tested (ibuprofen and naproxen), and, in fact, sweet cherries exhibited about 5% greater COX-2 inhibition than these medications. Similarly, data from the laboratory of Hou also indicate a significant COX-2 inhibitory effect of anthocyanin constituents found in sweet cherries and further demonstrate that these effects are related to down-stream inhibition of mitogen-activated protein kinase (MAPK)(Hou, 2005).

The anti-inflammatory effects of cherries have also been investigated in animal models of arthritis, a primary inflammatory disease affecting over 43 million Americans (CDC, 2006). In a study conducted by He at al, using induced arthritis model, male Sprague Dawley mice were fed 40, 20 or 10 mg/kg of total cherry anthocyanins daily in mouse chow for 28 days or standard un-enriched mouse chow (He, 2006). Anti-inflammatory response was assessed through the measurement of serum tumor necrosis factor alpha and prostaglandin E2 levels in paw tissue. Results suggested that, as expected, the induction of arthritis was successful as illustrated by elevated serum TNFα levels. Feeding at the highest dose of anthocyanins resulted in a significantly lower TNFα level as compared to standard feeding, but lower doses were not therapeutic in this regard. PGE2 levels in paw tissue samples showed a significant rise with induction of arthritis and a dose-responsive effect of anthocyanin

feedings, in that while all doses reduced PGE2 levels as compared to standard feeding, this effect was greatest at the 40 mg/kg dose, followed by the 20 mg/kg dose and finally the 10 mg/kg dose. This study provides preliminary evidence of the potential role of cherries in reducing inflammatory response in those with inflammation-related chronic illness. It is important to consider that extrapolation of the doses used in this mouse study would suggest that a 70 kg man would need to eat 2800 mg anthocyanins daily for several weeks. This is the equivalent of over 400 cups of fresh tart cherries or 35 cups of sweet cherries daily, amounts unobtainable in human feeding studies. While bioavailability differences are likely variable across species, the only real way to assess dose-response would be a well-designed cherry feeding study with standardized exposure in terms of anthocyanin content (dose).

In a pilot study investigating the effects of sweet cherry consumption on inflammatory markers in humans, 18 healthy adults (age 45-61 years) were fed 280 grams or approximately 2.5 cups of sweet cherries daily for 4 weeks (Kelley, 2006). Inflammation was assessed by repeat measures of serum C-reactive protein (CRP) levels. CRP levels were reduced by 8 and 25% at Day 14 and 28 cherry feeding, respectively as compared to CRP levels prior to daily cherry consumption, indicating a significant drop only at the 28 day time point ($P < 0.05$). After cherry feeding was discontinued, CRP levels rose in the study population by an average of 10% in 34 days, although this rise did not reach statistical significance. Interleukin 6 levels were not changed in relation to sweet cherry intake and this was also true for several *ex vivo* secretion levels of IL-6 and TNFα. While of interest, these results need to be replicated. It would also be of value to evaluate the anti-inflammatory effects of sweet cherry feeding in study populations demonstrating baseline elevations in inflammatory status (obese, those with chronic inflammation-related illness) and to incorporate a broader range of inflammatory outcome markers.

In relation to the anti-inflammatory properties of sweet cherry components, cherries have been investigated in relation to pain control. Evidence suggesting a role of dietary constituents in reducing pain is expanding (Tall, 2004). In a novel study testing the role of tart cherry anthocyanins in pain control in rats, Tall and colleagues showed that anthocyanins provided at a dose of 400 mg/kg resulted in a significant reduction in paw withdrawal from heat-induced pain stimuli and von Frey filament exposure.

Gout, an inflammation associated disease which affects over 4.3 million Americans, particularly those who are male and obese, manifests as a consistent and significant elevation in plasma urate levels. Thus, to assess the pain-reducing potential of cherries, a pilot study was conducted among ten healthy women, age 22-40 years who were fed a single dose of 280 grams of de-pitted sweet cherries (Jacob, 2003). Blood samples to assess urate levels were collected before cherry feeding and 1.5, 3 and 5 hours post feeding. Results demonstrated a significantly lower mean serum urate level 5 hours after cherry feeding, a protective effect note shown with grape, strawberry or kiwi fruit feeding. This single-dose cherry feeding did not modify plasma CRP or nitric oxide as was suggested with longer term feeding (28 days) (Kelley, 2006).

Alzheimer's disease

Flavonoids and procyanidin compounds have been shown to reduce oxidant stress and β-amyloid production and thus may indirectly reduce the risk for Alzheimers disease (Yoshimura, 2003; Heo, 2004). Only recently has there been published evidence of the potential role of sweet cherry phenolic compounds in protecting neuronal cells involved in neurological function. The phenolics in sweet cherries include both quercetin and hydroxycinnamic acid as well as anthocyanins. In a recent cell culture study in which neuronal cells were exposed to a variety of sweet and tart cherry phenolic compounds, total phenolics and predominantly anthocyanins, demonstrated a dose-dependent reduction in oxidant stress (Kim, 2005). This preliminary evidence should provide impetus for further

investigation into the potential protective effects of sweet cherry bioactive compounds in reducing risk for or morbidity related to Alzheimers disease.

Sleep and Jet Lag

Melatonin is a hormone produced by the pineal gland that in addition to antioxidant activity also plays a role in promoting healthy circadian rhythm and thus promoting healthy sleep patterns. Cherries are one plant food source of melatonin and melatonin levels have been estimated to be higher in tart cherries as compared to sweet cherries. In a study of melatonin content in Egyptian foods, melatonin levels in select grains ranged from 87 to 187 ng/100 grams food; concentrations in fruits such as pomegranates and strawberries were much lower ranging from 13-29 ng/100 gram (Badria, 2002). In a study of two tart cherry varieties, Montmorency cherries had an estimated melatonin content of 1.35 μg / 100 gram serving while Balaton cherries averaged 0.2 μg/100 gram serving, suggesting that variety is an important determinant of melatonin content (Burkhardt, 2001).

Melatonin supplementation appears to be efficacious in reducing jet lag (Herxheimer, 2002; Suhner, 2001), although not consistently (Spitzer, 1999). One explanation for inconsistent results in published studies may be that supplementation is most efficacious in people with demonstrated low excretion of melatonin during sleep as was demonstrated in a double-blind, placebo-controlled study (Leger, 2004). Dosing levels used in clinical intervention trials for sleep or jet lag generally range between 2 and 5 mg/day. Thus, while sweet cherries hold potential to enhance sleep and reduce jet lag related to the available melatonin, it is not likely that usual intake levels required to replicate doses used in clinical trials can be attained or sustained. Again, in combination with other behavioral approaches to promote sleep or reduce jet lag, sweet cherry intake in usual amounts could prove to be useful.

PRODUCTION AND CONSUMPTION

Although the U.S. has historically been the largest exporter in the world cherry market, currently the world production of cherries is the highest in Turkey, followed by the U.S. and Iran (FAO, 2006). Annually more than 50,000 tons of sweet cherries and 10,000 tons of tart cherries are exported from the U.S. The total production area for all cherries produced in the U.S. is reportedly 31,677 ha (producing 253,286 tons in 2005), in which the production area for sweet cherry increased almost linearly over 10 years, while that of tart cherry decreased (USDA Census, 2002). The State of Washington records the highest production of sweet cherries in the U.S. (150,000 ton; USDA NASS, 2006).

The majority of sweet cherry production is for fresh consumption with 40% processed as brined, canned, frozen, dried or juice. In contrast ninety-nine percent of tart cherries are processed primarily for use in cooking and baking. Limited data are available to estimate sweet cherry intake in the U.S., although it is clear that the majority of sweet cherry consumption is fresh and that there is significant seasonal differences in intake. Epidemiological studies to assess the relationship between cherry intake and health outcomes are limited by the lack of assessment of cherry intake.

Factors affecting Nutrient Content or Bioavailability of Bioactive Food Components

Ripening and Environment

Anthocyanin content of cherries, a major form of antioxidants in cherries, increases exponentially as the fruit ripens. In addition to the accumulation of anthocyanins, there is a decrease in chlorophyll, and changes in other chemical constituents that occurs during the cherry ripening process.

Serrano et al. (2005) reported changes in concentrations and activities of antioxidants of sweet cherry at 14 different stages of ripeness. They analyzed color, texture, sugars, organic acids, total antioxidant activities, total phenolic compounds, total anthocyanins, and ascorbic acid concentrations. Total anthocyanins increased exponentially from stage 8 and reached the maximum value at stage 14 (63.26 mg cyanidin equivalent activity per 100 g fresh sample). Total antioxidant activity (TAA) decreased from stage 1 to stage 8, and increased again from stage 8 to stage 12, and coincided with dynamics in total phenolic compound concentration and the accumulation of anthocyanins. TAA reached the maximum activity at stage 14 (50.03 mg of ascorbic acid equivalent activities per 100 g fresh sample). Harvesting sweet cherries at stage 12 of ripening, when fruit reaches maximum size would develop the highest organoleptic, nutritional and functional quality attributes.

Effects of harvest year and harvest time on anthocyanin concentrations have been reported (Poll et al., 2003). Large differences in the concentration of soluble solids, acid as well as anthocyanin were found between harvests of ‘Stevnsbær’ tart cherry harvested 7-10 times per year for 3 years . The highest levels of these quality attributes were found in the year characterized by higher temperature and greater solar radiation. The cyanidin-3-glucosid equivalent anthocyanin concentrations in the harvested cherry juice varied from as low as 500 mg/L to as high as 2300 mg/L.

Ultra violet light (UV-light) has reportedly increased anthocyanin concentrations of grapes (Kubota and Tsuchiya, 2001), apples (Arakawa et al., 1986) and sweet cherries (Arakawa, 1993). In cherries, a more significant increase of anthocyanin concentration was observed for postharvest cherries irradiated with UV-B (280-320 nm) than those with UV-A (320-400 nm) (Arakawa, 1993). Under a UV fluorescent lamp (1.3 W m^{-2} irradiance), ‘Satonishiki’ sweet cherries accumulated twice as much anthocyanin as those under a white fluorescent light (4.0 W m^{-2}) after 72 hours of irradiation. These data suggest that a small amount of UV light in the environment during cherry ripening has a significant effect on the resulting accumulation of anthocyanins. The use of shade materials and bird screen has the potential to reduce the UV light compared with that under unshaded or unscreened conditions. The cherries grown under shade or screen may have lower anthocyanin concentrations, although there is limited information available on the effects of pre-harvest conditions on the bioactive compositions and concentrations in harvested cherry fruit.

Processing

Bioactive compounds of fresh fruits and vegetables change according to pre-harvest conditions (including cultivation procedures, harvesting timings, and climate conditions), and post-harvest conditions (including storage conditions and shipping conditions). Sweet cherries contain approximately 1500 mg total phenols per kg fresh weight, with the phenols comprised mainly of hydroxycinnamates, anthocyanins, flavin-3-ols (catechins), and flavonols (Gao and Mazza, 1995; Goncalves, 2004). Considering cherries are often stored at 2-5˚C for several weeks during postharvest before reaching the consumers, information on changes in the phenolic bioactive compounds during select storage conditions is imperative.

Effects of storage temperature and duration on sweet cherry bioactive compounds (phenolics) were reported by Goncalves et al. (2004). The levels of phenolics and anthocyanins varied among cultivars and storage conditions. Storage at 15˚C increased the concentration of cynanidin-3-rutinoside (anthocyanin), while 2˚C caused changes specific to cultivars. Extracts of fresh harvested cherries exhibited significantly higher antioxidant activities than stored samples.

Comparisons in anthocyanins and polyphenolic compositions of fresh and processed cherries has been reported by Chavanalikit and Wrolstad (2004). More than 75% of anthocyanins in frozen Bing cherries were destroyed after 6 months of storage at -23˚C. Storage at -70˚C caused less degradation in anthocyanins and total phenolics. ORAC and FRAP assays indicated a decrease in antioxidant activity after 3 or 6 months of storage at -23˚C, but an increase after storage at -70˚C. In

their studies of canned fruit, they found about half of the anthocyanins and polyphenolics were leached from the fruits into the syrup with little total loss per total can.

Changes of anthocyanin concentrations after processing fresh fruits to jams are reported for four cultivars of tart cherries by Kim and Padilla-Zakour (2004). All cultivars showed a significant decrease in anthocyanin concentrations (21-24% of the original level of the fresh fruits) due to the canning process of heating under high acid and sugar concentrations, although good retention was observed for the total phenolics and antioxidant capacity of the canned product.

CONSIDERATIONS FOR FUTURE RESEARCH

Dietary Measurement.

In addition, efforts to quantify cherry intake in the context of epidemiological research is warranted. While cherry intake has historically been seasonal in nature, with expanded access through importation from South America and Turkey, Americans can enjoy cherries almost year-round. More frequent intake and year-round access suggest that cherries should be considered for inclusion on food frequency questionnaire instruments commonly employed to assess diet-disease associations in large study populations.

Biomarkers of Exposure.

In addition to more accurately assessing reported dietary intake of cherries, biomarkers of cherry exposure are needed to assess potential health related effects of cherry intake, especially given the variability in nutrient and bioactive food component composition in relation to cherry cultivar, ripening, processing, etc. Identifying the most reliable and valid biomarkers of intake in humans will contribute significantly to advancing the testing of hypotheses in this area. Scientifically acceptable biomarkers need to be valid, correlate significantly with dietary intake, be reliable and utilize biological samples which are easily collected from free-living people.

Need for Human Feeding Studies.

While the current state of the evidence suggests that eating sweet cherries holds potential for improving overall health, more research is essential to clearly understanding the role of cherry consumption in reducing chronic disease risk, particularly in relation to human studies and establishing dose-specific guidelines. The mechanistic evidence exists to suggest that specific bioactive food components in sweet cherries can modulate oxidant stress and inflammation. This evidence warrants further scientific investigation regarding the role of sweet cherries in health. Although isolation of key bioactive food components to establish a specific dose-response would be one approach, in all likelihood it is the synergy among bioactive food components found in sweet cherries such as ascorbic acid, carotenoids and anthocyanins that results in the health-promoting effects realized from consuming the whole fruit.It is critical that the mechanistic research findings be further substantiated through the implementation of well designed human cherry feeding studies using fruits produced, harvested, stored, and distributed under standardized conditions as both pre-harvest and post-harvest conditions can significantly affect the concentrations of bioactive food components.

CONCLUSIONS

Sweet, fresh cherries and several cherry products are important sources of nutrient and bioactive food components in the human diet. Epidemiological studies assessing the role of nutrients and phytochemicals common to cherries (such as fiber, polyphenols or carotenoids) and specific health

outcomes provide indirect evidence for the role of cherries in health promotion. The health-promoting effects of cherries have been demonstrated in select basic and animal studies; however, human intervention trials remain sparse. Such feeding studies should include some assessment of dose-response under standardized cherry production methods in order to more fully understand the optimal dose of cherry intake necessary to promote modulation of disease-specific biomarkers.

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Prevention.com
Smart Ways to Live Well

Sweet Protection from Heart Disease

Snack on cherries to lower your risk of heart attack and stroke.

by Holly McCord, RD, with Gloria McVeigh

Though gout brings to mind Dickensian characters nursing swollen tootsies, its toxic source--high uric acid levels in the blood--portends future heart attacks and strokes. New preliminary research found that after 10 women ate 45 sweet cherries, their blood uric acid levels plummeted by 15%.

"It seems the anthocyanins that impart the lovely red color to cherries decrease blood urate, so they may help lower heart attack and stroke risk," says Robert A. Jacob, PhD, author of the USDA/University of California study. Jacob says canned or dried cherries, tart cherries, and cherry juice contain the same anthocyanins as fresh sweet cherries. One serving a day should have some benefit.

Holly McCord is Prevention's *former Nutrition Editor. Gloria McVeigh is the Nutrition News Editor.*

BACK TO TOP

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Got arthritis? Fresh cherries may help

ARS News Service

Plump, juicy Bing cherries, eaten fresh, may help people who suffer from the pain of gout or other forms of arthritic inflammation. That's according to preliminary results from research at the Agricultural Research Service's Western Human Nutrition Research Center in Davis, Calif.

The 10 healthy women, aged 22 to 40, who volunteered for the first phase of this research ate a special breakfast of 45 fresh, pitted Bing cherries.

ARS chemists Robert A. Jacob (now retired) and Darshan S. Kelley collaborated with university scientists in that preliminary study and, recently, in a more extensive follow-up investigation.

The experiments are among the first to track anti-inflammatory effects of fresh Bing cherries in carefully controlled tests with healthy volunteers. That's in contrast to previous studies, conducted elsewhere, in which scientists analyzed extracts from sweet or tart cherries in the laboratory.

Jacob and Kelley found that levels of uric acid — a compound the body uses to form painful urate crystals during a gout attack — decreased significantly in volunteers' blood (plasma) over the five hours after they ate the Bing-cherry breakfast. And, levels of urate removed from their bodies in urine increased over those five hours.

On the Internet:

www.ars.usda.gov/is/AR/archive/may04/cherry0504.htm. The grower-sponsored California Cherry Advisory Board, Lodi, Calif., helped fund the research.

CAP PRESS 5/14/04

Fresh Cherries May Help Arthritis Sufferers

Fresh Bing cherries.
(K11182-1)

Arthritis hurts. But fresh cherries may help.

Results of a preliminary study by ARS scientists and their university colleagues suggest that some natural compounds in plump, juicy Bing cherries may reduce painful arthritic inflammation. Eating cherries may also help lessen the severity of other inflammatory conditions, such as cardiovascular disease or cancer.

Cherries already have a reputation for fighting inflammation. So what's new about the ARS study?

"Our test is among the first to track anti-inflammatory effects of fresh Bing cherries in a controlled experiment with healthy volunteers," says chemist Robert A. Jacob, who led the investigation. Jacob is now retired from the ARS Western Human Nutrition Research Center in Davis, California.

In previous studies at other laboratories, scientists analyzed extracts from sweet or tart cherries in vitro to learn more about the fruit's potential health-promoting properties. In contrast to these test-tube experiments, the California study is apparently the first to test key inflammatory disease indicators, or markers, in blood samples from healthy volunteers who were fed precise amounts of fresh Bing cherries. Reported in a 2003 issue of the *Journal of Nutrition*, the California investigation paved the way for a recent followup study at the Davis center.

Chemist Darshan Kelley (left) and Adel Kader, professor at the University of California, Davis, examine and weigh cherries.
(K11171-1)

Life—A Bowl of Cherries?

Imagine being asked to eat a bowlful of 45 fresh, pitted Bing cherries for breakfast. Ten healthy women, aged 22 to 40, agreed to do that for the California scientists' preliminary study. Volunteers were instructed not to eat strawberries or other fruits and vegetables, or to drink tea or red wine, for the 2 days before the cherry breakfast. These foods are high in antioxidants, thought to fight inflammation. "They could have interfered with our ability to determine the specific effects of the Bing cherry antioxidants," explains Jacob.

"Our main focus in this study was gout, a very painful form of arthritis," says co-investigator Darshan S. Kelley, a chemist at the nutrition center. "During gout attacks, crystals of a naturally occurring chemical, uric acid, accumulate in joints—commonly in the toes—and cause pain. Urate in blood plasma is a precursor of these uric acid crystals. So, we closely measured volunteers' levels of plasma urate.

Danise Gonzalez, a registered nurse with ARS's Western Human
Nutrition Research Center,

"We also indirectly measured the amount of urate that was moved out of the body in urine. We took blood plasma and urine samples before the volunteers ate the cherry breakfast and at intervals of 1-1/2, 3, and 5 hours afterward."

Volunteers' plasma urate levels decreased significantly over the 5 hours after their meal of cherries. Levels of urate removed from the body in urine increased over those 5 hours.

These urate results strongly suggest that cherries can play an important role in fighting gout. So do the results from the scientists' assays of some other indicators of inflammation. Significant changes in the levels of markers are an indication of a healthy immune system at work, attacking

completes a blood draw on a participant in a study of the potential benefit of cherries for inflammation and arthritis.
(K11174-1)

inflammation. Markers monitored included C-reactive protein, nitric oxide, and tumor necrosis factor alpha.

C-reactive protein, produced by the liver, increases rapidly during inflammation, such as during a gout attack. In a healthy body, blood (serum) levels of C-reactive protein are extremely low.

A study participant prepares to eat cherries for breakfast in a study designed to evaluate the fruit's potential benefit for inflammation and arthritis.
(K11184-1)

Another reliable sign of inflammation: the unwanted increase in nitric oxide. This biochemical is thought to play a role in damaging arthritic joints. The third marker, tumor necrosis factor alpha, is secreted in greater quantities when the body is fighting tumors that may induce inflammation. As is true for C-reactive protein, a healthy body that isn't fighting an inflammation has very little of this marker.

At the 3-hour monitoring interval, C-reactive protein and nitric oxide were somewhat lower than at the start of the study. "Even though these levels were not significantly lower, the trend was in the right direction and so is of interest," notes Kelley.

Unexpectedly, the scientists found no change in levels of tumor necrosis factor alpha. That's in contrast to a previous study, conducted elsewhere, in which natural compounds in fruits and vegetables were found to decrease levels of this marker. But the trends toward decreases in the other two markers do agree with results of other scientists' earlier, in vitro studies of cherry extracts.

Jacob and Kelley collaborated with chemists Giovanna M. Spinozzi and Vicky A. Simon of the nutrition center; chemist Ronald L. Prior, who is with ARS at Little Rock, Arkansas; and research associate Betty Hess-Pierce and professor Adel A. Kader, of the University of California, Davis.

A Month of Fresh Cherries

The follow-up study, conducted in 2003, involved more people, more cherries, and a greater array of inflammatory-response markers. Eighteen women and two men—aged 22 to 40—participated in the 64-day investigation.

Many of the new volunteers began the study with elevated C-reactive protein levels. "That made it easier to detect any decline in C-reactive protein levels as the study progressed," says Kelley. "We're particularly interested in this protein because a recent major study indicated that it's more reliable than cholesterol as a predictor of cardiovascular disease.

"This group ate the same daily amount of fresh Bing cherries as our earlier volunteers. But we asked them to eat the cherries throughout the day instead of just at breakfast." The volunteers did that for 28 consecutive days. The researchers are now analyzing blood samples.

The grower-sponsored California Cherry Advisory Board helped fund the research. Final results should be available later this year. Then we'll know more about the health benefits of this sweet treat.—By **Marcia Wood,** Agricultural Research Service Information Staff.

This research is part of Human Nutrition, an ARS National Program (#107) described on the World Wide Web at www.nps.ars.usda.gov.

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"Fresh Cherries May Help Arthritis Sufferers" was published in the **May 2004** issue of *Agricultural Research* magazine.

Consumption of Cherries Lowers Plasma Urate in Healthy Women[1,2]

(Manuscript received 3 January 2003. Initial review completed 30 January 2003. Revision accepted 28 February 2003.)

Robert A. Jacob,[3] Giovanna M. Spinozzi, Vicky A. Simon, Darshan S. Kelley, Ronald L. Prior,* Betty Hess-Pierce† and Adel A. Kader†

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ABSTRACT To assess the physiologic effects of cherry consumption, we measured plasma urate, antioxidant and inflammatory markers in 10 healthy women who consumed Bing sweet cherries. The women, age 22–40 y, consumed two servings (280 g) of cherries after an overnight fast. Blood and urine samples were taken before the cherry dose, and at 1.5, 3 and 5 h postdose. Plasma urate decreased 5 h postdose, mean ± SEM = 183 ± 15 μmol/L compared with predose baseline of 214 ± 13 μmol/L ($P < 0.05$). Urinary urate increased postdose, with peak excretion of 350 ± 33 μmol/mmol creatinine 3 h postdose compared with 202 ± 13 at baseline ($P < 0.01$). Plasma C-reactive protein (CRP) and nitric oxide (NO) concentrations had decreased marginally 3 h postdose ($P < 0.1$), whereas plasma albumin and tumor necrosis factor-α were unchanged. The vitamin C content of the cherries was solely as dehydroascorbic acid, but postdose increases in plasma ascorbic acid indicated that dehydroascorbic acid in fruits is bioavailable as vitamin C. The decrease in plasma urate after cherry consumption supports the reputed anti-gout efficacy of cherries. The trend toward decreased inflammatory indices (CRP and NO) adds to the in vitro evidence that compounds in cherries may inhibit inflammatory pathways. J. Nutr. 133: 1826–1829, 2003.

KEY WORDS: • cherries • gout • humans • anti-inflammatory • fruit

In addition to providing essential vitamins, minerals and dietary fiber, fruits contain phytochemicals that may lower the risk of cancer, heart disease and other chronic diseases. Both sweet and tart cherries are rich in antioxidants, including anthocyanins (responsible for red skin and flesh color), catechins, chlorogenic acid, flavonal glycosides and melatonin. Anthocyanins, cyanidin and hydroxycinnamates isolated from tart or sweet cherries inhibited oxidation of isolated human LDL and model liposomes to an extent comparable to vitamin E and BHT (1–3). Anthocyanins extracted from cherries have also shown anti-inflammatory properties, via inhibition of cyclooxygenase (COX)[4] activities (2,3) and scavenging of the reactive nitric oxide (NO) radical (4). In activated macrophages, anthocyanins and other phenolics inhibit NO production and modulate tumor necrosis factor (TNF)-α secretion (5,6).

Consumption of cherries and cherry products has been reported to be health promoting, particularly to alleviate arthritic pain and gout (7). Clinical case reports of three patients with gout showed that consumption of 227 g of cherry products daily for 3 d to 3 mo reduced plasma urate to normal levels and alleviated attacks of gouty arthritis (7). It is not known what compounds in cherries might be responsible for these alleged actions. Moreover, the putative anti-gout and anti-inflammatory properties of cherries have not been assessed in controlled experimental studies. The present study was conducted to determine the extent of these effects in healthy women consuming an acute dose of Bing sweet cherries.

SUBJECTS AND METHODS

Subjects and study design. The clinical portion of the study was conducted in May 2002, during California's fresh cherry season, at the USDA Western Human Nutrition Research Center (WHNRC), 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 and urine tests including a complete blood cell count with leukocyte differential, clinical chemistry panel, urinalysis and tests for infectious disease. Candidates were excluded if they were in poor health, obese (body mass index >30 kg/m^2), regularly used nutritional supplements, medications, alcohol or recreational drugs. The ten women accepted into the study were nonsmokers, age 22–40 y (mean ± SD = 29.9 ± 6.1 y) and primarily Caucasian. The study was approved by the Human Subjects Review Committee of the University of California, Davis. All subjects signed informed consent before entering the study.

To partially standardize and limit intake of antioxidants before the experimental cherry dose, subjects were asked to refrain from consuming fruits and vegetables or their juices, tea or wine for 2 d before the cherry dose. Fresh sweet Bing cherries were obtained from O.G. Packing, Stockton, CA and were stored at 4°C until they were consumed. The subjects consumed 280 g of depitted cherries (about 45 cherries) after an overnight fast and were required to consume all

[1] Supported in part by the United States Department of Agriculture, Agricultural Research Service, and the California Cherry Advisory Board, Lodi, CA.
[2] Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.
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[4] Abbreviations used: AA, ascorbic acid; COX, cyclooxygenase; CRP, C-reactive protein; DHA, dehydroascorbic acid; FRAP, ferric reducing ability of plasma; NO, nitric oxide; ORAC, oxygen radical absorbing capacity; TEAC, Trolox equivalent antioxidant capacity; WHNRC, Western Human Nutrition Research Center.

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of the cherries within 10 min. Blood and urine samples were obtained before the dose, and at 1.5, 3 and 5 h postdose. Subjects emptied their bladder for the predose urine collection, and all urine was collected between each blood draw. Subjects were allowed to leave the clinical unit after the 1.5- and 3-h postdose blood draws but were required to return within 10 min of the next scheduled blood draw, and avoid consumption of any food or drink except from a 237-mL bottle of water given after the 1.5-h draw. The subjects were scheduled over 6 d and a 70-g portion of the cherries available was taken on each of the 6 d and frozen at −70°C until analysis for antioxidant and polyphenol content.

For comparison purposes, plasma urate values from a previous (unpublished) study on antioxidant capacity of fruits are included herein. The study design was similar to the present study, i.e., two servings each of red "crimson seedless" grapes (280 g), "Seascape" strawberries (300 g) and "Hayward" kiwifruit (300 g) were consumed 1 wk apart by seven healthy women, 18–40 y old, and blood samples were drawn over the next 5 h.

Sample collection and laboratory methods. Blood was drawn by venipuncture into evacuated tubes with EDTA and heparin anticoagulants. The blood was immediately processed to separate red cells in a refrigerated centrifuge and aliquots of the plasma were frozen at −70°C for later analysis. An aliquot of EDTA plasma was treated with an equal volume of meta-phosphoric acid (100 g/L) and the protein-free supernatant frozen at −70°C for later determination of ascorbic and uric acids by HPLC with electrochemical detection (8). A portion of the heparinized plasma was treated with an equal volume of 0.5 mol/L perchloric acid and the protein-free supernatant was frozen at −70°C for later determination of antioxidant capacity.

Urine was collected in tared containers and the total weight of the urine collection was recorded. After mixing of the urine, dipstick urinalysis was completed (Ames Diagnostics, Indianapolis IN), and aliquots of the urine were frozen at −20°C for later determination of creatinine and urate.

Urine urate was determined by a colorimetric peroxidase/uricase procedure utilizing a 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system applied to a centrifugal analyzer (9). Plasma and urine creatinine were determined by the Jaffe picric acid spectrophotometric method adapted to the Hitachi 902 automated analyzer (Roche Diagnostic, Indianapolis, IN). Antioxidant capacities were determined in the cherries and blood plasma by the hydrophilic and lipophilic oxygen radical absorbing capacity (ORAC) methods (10,11), the spectrophotometric radical cation decolorization method utilizing 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate), also known as the Trolox equivalent antioxidant capacity (TEAC) method (12) and the ferric reducing ability of plasma (FRAP) method (13).

Plasma C-reactive protein (CRP), TNF-α and NO were measured as inflammatory markers. CRP was measured by a high sensitivity enzyme immunoassay (Biocheck, Burlingame, CA). Plasma TNF-α was measured using the Quantikine high sensitivity TNF-α colorimetric immunoassay kit, and plasma NO was measured using the Total Nitric Oxide colorimetric assay kit (R&D Systems, Minneapolis MN).

Polyphenols 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 (14). Total phenolics were determined in the polyphenol extract using a modified spectrophotometric Folin-Ciocalteu method (15). Ascorbic and dehydroascorbic acids were extracted from cherries with a citric acid buffer and determined by HPLC with UV diode-array detection (16).

Statistical analysis. Data were analyzed using Instat 3.0 (GraphPad Software, San Diego CA). Descriptive statistics were computed for study measures at baseline (predose) and the three postdose time points. Urinary urate was normalized to creatinine concentrations. Repeated measures ANOVA with Student-Newman-Keuls adjustment for multiple comparisons was used to determine the effect of the cherry dose over the entire study period, from baseline to 5 h postdose. Paired t tests or Wilcoxon signed-ranks tests were used to compare specific postdose values with baseline, including Bonferroni adjustment of probability levels for multiple comparisons. Results are presented as mean ± SEM. Differences were considered significant for the two-tailed P-value < 0.05.

RESULTS

Hydroxycinnamates comprised the largest class of phenolics in the cherries ingested, representing ~42% of the total phenolics of 163 mg/100 g (**Table 1**). The next largest fraction of phenolics was anthocyanins at 23%. Only dehydroascorbic acid (DHA), the oxidized form of vitamin C, was detected in the cherries. No HPLC peaks were detected for the reduced form, ascorbic acid (AA).

Plasma urate decreased significantly over the 5-h period after cherry consumption (ANOVA), and the concentration at 5 h postdose was significantly lower than at baseline (**Table 2**). Urinary urate, expressed per mmol creatinine, increased over the 5 h postdose and at each postdose sampling time compared with baseline. After similar doses of grapes, strawberries or kiwifruit, plasma urate concentrations did not change over time, nor were any postdose concentrations significantly lower than those at baseline.

Among inflammatory biomarkers, plasma TNF-α did not change after cherry consumption. Plasma CRP and NO did not decrease over the entire 5-h period (ANOVA), but both were marginally decreased ($P < 0.1$) at 3 h postdose compared with baseline, by Wilcoxon's signed-ranks test (CRP) and paired t test (NO) (Table 2). The plasma CRP data were not normally distributed because values for one subject were well above the normal range of 0.1–8.2 mg/L (17). The subject's baseline value of 22.9 mg/L was 2.7 SD above the mean and declined 44% to 12.9 mg/L at 3 h postdose.

Among antioxidant capacity measures, the hydrophilic ORAC and TEAC measures did not differ after cherry consumption, lipophilic ORAC increased and FRAP decreased at all postdose sampling times. Plasma ascorbic acid increased significantly at 1.5 and 3 h postdose. Plasma creatinine decreased significantly at 1.5 and 5 h postdose, and marginally ($P = 0.07$) at 3 h postdose. Plasma albumin was unchanged throughout.

DISCUSSION

Fruits contain a wide variety of phytochemicals that are known or suspected to provide health benefits, yet most phytochemicals in fruits have not been studied for their effect on

TABLE 1

Concentrations of antioxidant substances in Bing sweet cherries[1,2]

Substance measured	Concentration
	mg/100 g fresh weight
Hydroxycinnamates	67.9 ± 4.0
Procyanidins	21.7 ± 2.5
Flavanols	34.8 ± 3.9
Anthocyanins	38.0 ± 3.6
Total phenolics	163 ± 9
Vitamin C[3]	18.4 ± 2.3
Antioxidant capacity (TEAC), *µmol TE/100 g*	211 ± 8
Antioxidant capacity (FRAP), *µmol/100 g*	170 ± 2

[1] Values are mean ± SEM, $n = 5$ batches of cherries.

[2] TEAC, Trolox equivalent antioxidant capacity; FRAP, ferric reducing ability of plasma.

[3] As dehydroascorbic acid.

TABLE 2

Plasma and urine biomarkers in healthy women before and after cherry consumption[1,2,3]

Biomarker	Baseline	1.5 h	3 h	5 h
Plasma urate, *μmol/L*				
Cherries†	214 ± 13	221 ± 22	203 ± 13	183 ± 15*
Grapes	278 ± 25	263 ± 26	257 ± 23	260 ± 21
Strawberries	286 ± 25	280 ± 20	277 ± 25	262 ± 29
Kiwifruit	285 ± 28	256 ± 21	257 ± 23	281 ± 19
Urinary urate, *μmol/mmol creatinine*	202 ± 13	278 ± 29*	350 ± 33*	260 ± 17*
Plasma				
C-reactive protein, *mg/L*	4.29 ± 2.18	ND	3.07 ± 1.26	3.59 ± 1.59
Nitric oxide, *μmol/L*	37.4 ± 5.2	ND	31.1 ± 2.9	31.6 ± 2.1
ORAC (lipophilic),[4] *μmol TE/L*	531 ± 37	628 ± 37*	681 ± 24*	711 ± 27*
FRAP, *μmol/L*	454 ± 23	432 ± 21*	403 ± 14*	414 ± 21*
Ascorbic acid, *μmol/L*	65.4 ± 5.6	74.5 ± 5.6*	71.8 ± 6.0*	68.2 ± 5.2

[1] Values are means ± SEM, $n = 10$. * Different from baseline, $P < 0.05$. † Significant decrease over time, $P < 0.05$.
[2] Plasma urate concentrations of grapes, strawberries, and kiwifruit are for seven women in a separate but similar study (unpublished data) with the last time point at 4.5 h.
[3] Abbreviations: ND, no data; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing ability of plasma.
[4] Units are μmol Trolox equivalents/L.

human health. Polyphenolic flavonoids have been shown to provide antioxidant, anti-inflammatory, antithrombotic, and anticarcinogenic actions, which may reduce the risk of chronic diseases (18). Deeply colored cherries and berries contain a large amount of phenolic compounds, ~9 times the amount of vitamin C for the Bing sweet cherries ingested in the present study (Table 1). Cherries have a unique reputation for providing anti-gout and anti-inflammatory benefits; this is largely anecdotal and has not been confirmed in controlled nutrition studies. The present results support an anti-gout effect of cherries because the cherries provoked a significant decrease in plasma urate over 5 h postdose, whereas the other fruits produced no change (Table 2). Although the observed mean decrease (214 to 183 μmol/L or 14.5%) is within the lower range of normal (155–357 μmol/L) (17), it supports the claim that consumption of cherry products may benefit individuals who suffer from high levels of plasma urate and arthritic gout. By comparison, acute ingestion of milk proteins also lowered serum urate (19), whereas purine-rich foods (beef liver, haddock, soybeans) increased serum urate at 2–4 h postdose (20).

Data from the present study cannot definitively establish the mechanism whereby cherry consumption lowers plasma urate. Plasma urate is largely reabsorbed in the renal tubules after glomerular filtration, whereas plasma creatinine is cleared without reabsorption. The observed postdose increase in urinary urate per unit creatinine excretion and the decrease in plasma creatinine suggest that cherries may exert their urate-lowering effect by increasing the rate of renal glomerular filtration and/or reducing tubular reabsorption.

Biomarkers of the inflammatory response, plasma CRP, NO and TNF-α, were measured in the present study because of reports that consumption of cherries relieved the arthritic joint pain of gout (7), that anthocyanins and other phenolics inhibit NO and alter TNF-α production in activated macrophages (5,6), and that anthocyanins isolated from cherries inhibit the activity of the proinflammatory enzyme COX II in vitro (2,3). The trend toward decreased plasma CRP and NO 3 h after cherry consumption is consistent with previous in vitro evidence (2–5) and suggests that compounds in cherries may inhibit inflammatory pathways in vivo. Decreased in vivo NO production may reduce the progression of inflammatory arthritis because increased 3-nitrotyrosine concentrations found in rheumatoid arthritis patients have been cited as evidence that the NO radical plays a role in arthritic joint damage (21).

The constancy of plasma albumin values throughout indicates that postdose changes in urate and inflammatory markers were not due to changes in hemodilution or hydration status. That measures of plasma water-soluble antioxidant capacity were unchanged (hydrophilic ORAC and TEAC) or decreased (FRAP) after cherry consumption is not surprising because urate is the largest single contributor to plasma hydrophilic antioxidant capacity (22), and its concentration decreased after cherry consumption. The finding that lipophilic ORAC increased substantially is unexpected because most antioxidant compounds in cherries, e.g., phenolic glycosides and vitamin C are water-soluble compounds. However, reports that cherry and berry phenolics show strong antioxidant activity in phospholipid liposomes (1,2,23) indicate that these compounds are active in lipophilic as well as hydrophilic systems. Support for this includes findings that the less polar anthocyanin aglycone, cyanidin, has stronger antioxidant activity than its glycosides (2), and that flavonoids alter membrane fluidity by partitioning into the lipophilic core of model membranes (24). Melatonin may have contributed to the increase in lipophilic ORAC because it is more active than vitamin E as a lipophilic antioxidant (25) and occurs in "Balaton" and "Montmorency" cherries in amounts of 0.2 and 1.3 mg/100 g, respectively (26).

The finding that the cherries ingested contained only the oxidized form of vitamin C, dehydroascorbic acid (DHA), and not the reduced form, ascorbic acid (AA), is unusual among fruits. The average DHA content of 12 fresh fruits as a percentage of total vitamin C (AA + DHA) was 15.2%, with a range of 6–48% (27). Because care was taken to keep the cherries frozen until analysis, and acidic extraction buffers were used to preserve any AA, it is not likely that the DHA finding is due to artifactual oxidation of the vitamin C. Indeed, a small amount of AA standard added to a cherry homogenate was converted to DHA. This is likely due to oxidation of the AA in the cherries by phenoxyl semiquinone radicals and/or *o*-quinone metabolites formed from the reaction of polyphenols with polyphenol oxidases (28). That plasma AA increased significantly after consumption of cherries that contain only DHA (Table 2) argues against recent claims that DHA in fruits is poorly available as vitamin C (29,30) and is consistent

with evidence that DHA is absorbed in the small intestine and recycled into AA in vivo (31).

In conclusion, the decrease in plasma urate after cherry consumption supports the anti-gout reputation of cherries. The trend toward decreased plasma concentrations of the inflammatory markers CRP and NO adds to the in vitro evidence that compounds in cherries may inhibit inflammatory pathways. Further research is required to determine the potential of cherry and polyphenol consumption for inhibiting the inflammatory cascade and for improving the condition of individuals who are at risk or suffer from gout and arthritis.

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ORIGINAL ARTICLE

Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage

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Background: Numerous antioxidant and anti-inflammatory agents have been identified in tart cherries.
Objective: To test the efficacy of a tart cherry juice blend in preventing the symptoms of exercise induced muscle damage.
Methods: This was a randomised, placebo controlled, crossover design. Fourteen male college students drank 12 fl oz of a cherry juice blend or a placebo twice a day for eight consecutive days. A bout of eccentric elbow flexion contractions (2×20 maximum contractions) was performed on the fourth day of supplementation. Isometric elbow flexion strength, pain, muscle tenderness, and relaxed elbow angle were recorded before and for four days after the eccentric exercise. The protocol was repeated two weeks later with subjects who took the placebo initially, now taking the cherry juice (and vice versa). The opposite arm performed the eccentric exercise for the second bout to avoid the repeated bout protective effect.
Results: Strength loss and pain were significantly less in the cherry juice trial versus placebo (time by treatment: strength $p<0.0001$, pain $p = 0.017$). Relaxed elbow angle (time by treatment $p = 0.85$) and muscle tenderness (time by treatment $p = 0.81$) were not different between trials.
Conclusions: These data show efficacy for this cherry juice in decreasing some of the symptoms of exercise induced muscle damage. Most notably, strength loss averaged over the four days after eccentric exercise was 22% with the placebo but only 4% with the cherry juice.

Cyclo-oxygenase inhibitory flavonoids[1 2] and anthocynanins with high antioxidant and anti-inflammatory activities[1 3 4] have been identified in tart cherries, which are considered good sources of phenolic compounds. This has led to speculation that cherry consumption may be effective in alleviating symptoms in inflammatory conditions.[4] Anti-inflammatory drugs and food products containing antioxidant nutrients have been studied extensively in the treatment and prevention of exercise induced muscle damage and its associated symptoms. Some studies have shown efficacy with anti-inflammatory drugs[5–10] whereas others have not.[11–14] Similarly, studies examining the effect of antioxidants—for example, vitamins C and E—on the symptoms of muscle damage have yielded inconsistent results.[15–20] Discrepancies in the observed effects may, in part, be related to factors such as differences in muscle groups studied, differences in magnitude of muscle damage between studies, study sample sizes relative to interindividual differences in symptoms of damage, between group study designs versus crossover designs, whether the treatment was given before eccentric exercise, after eccentric exercise, or both, and differences in dosages.

Consumption of about 45 cherries a day has been shown to reduce circulating concentrations of inflammatory markers in healthy men and women.[21 22] Considering the natural anti-inflammatory and antioxidant capacity of tart cherries, it is plausible that cherry consumption before and after eccentric exercise may have a protective effect. Therefore the purpose of this study was to test the effect of a tart cherry juice blend taken before and after eccentric exercise on the symptoms of muscle damage.

METHODS

Sixteen men (mean (SD) age 22 (4) years, height 1.78 (0.76) m, weight 90 (18) kg) volunteered to participate in this study. The protocol was approved by the institutional review board, and all subjects gave written informed consent.

Protocol

Four days before eccentric exercise, subjects reported to the laboratory for baseline testing and to be provided with the cherry juice or placebo. Their arms were randomly assigned to a treatment or placebo trial and randomly assigned to perform the treatment or placebo trial first. Pain, muscle tenderness, relaxed elbow angle, and isometric elbow flexion strength were measured. Inclusion criteria were no elbow flexor pain, no upper extremity strength training in the past three months, and no history of elbow or shoulder injury. Subjects were also instructed not to take any anti-inflammatory or pain relieving drugs during the course of the study, not to seek any other treatment for any symptoms of muscle damage, and not to exercise their upper extremities during the study. They were given 12 oz bottles of placebo or cherry juice and instructed to drink one bottle in the morning and the other in the evening for the next eight days. On the fourth day, subjects returned to the laboratory and performed a bout of eccentric elbow flexion contractions. On each of the following four days, pain, muscle tenderness, relaxed elbow angle, and strength were assessed. Two weeks after the initial baseline testing (six days after the end of the first trial), subjects returned to the laboratory and the protocol was repeated on the contralateral arm with either the placebo or cherry juice provided, as determined by previous randomisation. Subjects were scheduled to attend the laboratory for data collection at the same time each day for both the exercise session and four days of follow up data collection.

Treatment and placebo drinks

The cherry juice blend was prepared by mixing freshly prepared tart cherry juice with commercially available apple juice in a proprietary ratio (Cherrypharm Inc, West Hartford, Connecticut, USA). Frozen tart cultivar Montmorency cherries were used to prepare the cherry juice following standard procedures that simulate industrial processing. The blended juice was pasteurised by heating it to 85℃, hot packed into

CCAB/WSFC SAB Mtg (Mar 09) Page 52

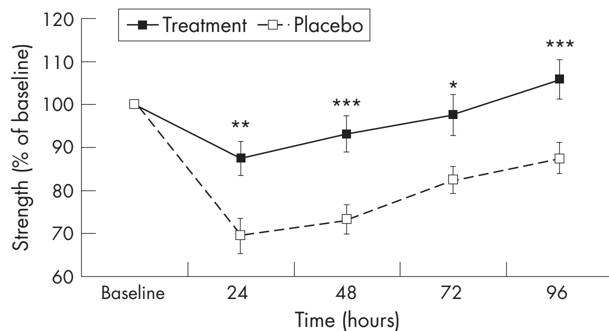

Figure 1 Isometric elbow flexion strength (expressed as a percentage of baseline strength) after eccentric exercise. Displayed values are averaged across all three test angles. Time by treatment $p<0.0001$; *$p<0.05$, **$p<0.01$, ***$p<0.001$ pairwise comparisons with Bonferroni corrections for treatment versus placebo values. Values are mean (SE).

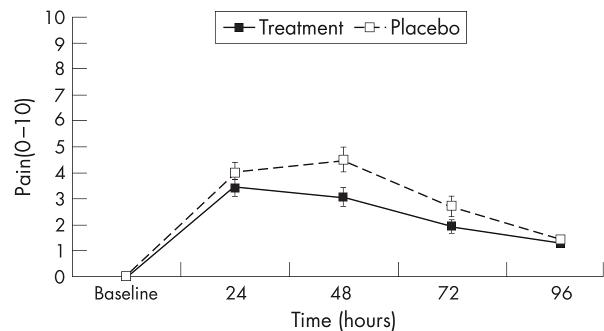

Figure 2 Subjective report of pain in the elbow flexors after eccentric exercise (0–10 scale). Time by treatment, $p = 0.017$. Values are mean (SE).

12 oz glass bottles with a three minute hold time to achieve commercial sterility, and then forced cooled in a water bath. One 12 oz bottle of the juice provided at least 600 mg phenolic compounds, expressed as gallic acid equivalents by the method of Singleton and Rossi,[23] and at least 40 mg anthocyanins, calculated as cyanidin-3-glucoside equivalents by the pH differential method described by Giusti and Wrolstad.[24] Each bottle contained the equivalent of 50–60 cherries.

The placebo was prepared by mixing unsweetened black cherry Kool-aid soft drink mix (Kraft Northamerica, Ryebrook, New York, USA; ingredients listed: citric acid, salt, calcium phosphate, red 40, artificial flavour, ascorbic acid, blue 1) with water in the proportion recommended by the manufacturer (about 2 g/l). Sugar was added to match the concentration of soluble solids in the cherry juice blend to a final concentration of 13 Brix (total percentage soluble solids by weight). The flavoured beverage was then pasteurised and bottled following the procedure used for the juice blend.

Eccentric exercise protocol

The exercise regimen for the induction of delayed onset muscle soreness consisted of 40 (2×20) maximal eccentric contractions of the elbow flexors using a modified preacher curl apparatus. In this study, the subject was instructed to apply maximal resistance through use of their elbow flexor musculature, while the investigator forced the subject's elbow into full extension. This was accomplished by pulling down on a lever that extended about 60 cm past an adjustable handle used to grip the lever by the subject. The added length of the lever past the handle provided a mechanical advantage over the subject's maximal flexion force, while requiring only limited effort to be exerted by the investigator. The subject's starting elbow angle position for each maximally resisted movement was full active elbow flexion (about 130° flexion). Subjects performed two sets of 20 maximal eccentric contractions, with a three minute rest period between sets. Each eccentric contraction lasted approximately three seconds, with 12 seconds of rest between actions.

Measurement of pain

Pain scores were obtained by asking subjects to verbally rate their overall discomfort during active elbow flexion and extension with activities of daily living on a scale of 0–10. A score of 0 indicated no discomfort whatsoever. A score of 10 indicated extreme pain and discomfort. Subjects were given a standard description for examples of daily activities which included brushing teeth, opening a bottle, driving a car, or opening doors.

Measurement of muscle tenderness

Muscle tenderness scores were assessed using a standard manual muscle myometer. Measurements were made just proximal to the distal tendon of the biceps brachii. All measurements are reported in Newtons (N). Force was applied via the probe through a 1 cm diameter head until the subject indicated pain or discomfort. At this point the force value (N) was recorded. Tenderness scores on the days after eccentric exercise were subtracted from baseline scores to provide a measure of tenderness, where zero equalled no tenderness. Previous studies have used a ceiling of 40 N for detecting muscle tenderness,[25 26] but it was felt that this obscured interindividual differences in tenderness sensitivity. For example, six subjects reported discomfort at less than 40 N at baseline on the treatment arm, and five subjects reported discomfort at less than 40 N on the placebo arm. Baseline tenderness was 47 (17) N on the treatment arm and 46 (15) N on the placebo arm. The system was calibrated daily using a balanced 1 kg mass, 2 kg mass, and 3 kg mass converted into Newtons (mass $\times$ 9.81 N).

Assessment of relaxed elbow angle

Elbow range of motion was assessed using a standard plastic goniometer (Lafayette Instrument, Lafayette, Indiana, USA) with the subject standing. The axis of the goniometer was placed over the lateral epicondyle of the elbow. The stationary arm of the goniometer was placed in line with the long axis of the humerus pointed at the acromion process. The movement arm was placed in-line with the long axis of the forearm. The placement locations of the goniometer axis, movement arm, and stationary arm were marked with permanent ink for consistency throughout trials.

Measurement of isometric elbow flexion strength

Subjects were tested on a modified, seated, arm curl (preacher) bench with the upper arm supported by a padded bench in about 45° shoulder flexion. Isometric strength was tested at three different elbow flexion angles: 130°, 90°, and 30°. The subjects grasped the handle attached to a movement lever mounted on the arm curl device. Force was recorded by a force transducer (model L-2352; Futek Inc, Irvine, California, USA) in series with chains attached to the test apparatus. Two trials were performed at each test angle with each contraction lasting three seconds, and 60 seconds rest between contractions. Peak strength values were recorded in Newtons, and the mean of the two trials served as the maximal isometric contraction strength score at that angle.

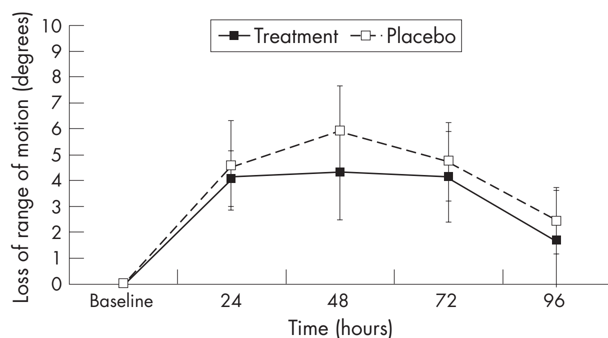

Figure 3 Changes in relaxed elbow angle after eccentric exercise. Time by treatment, p = 0.85. Values are mean (SE).

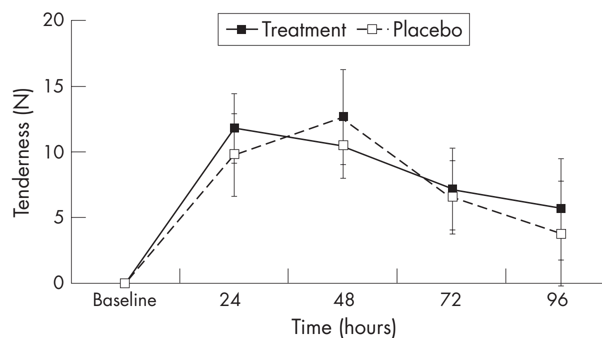

Figure 4 Changes in muscle tenderness after eccentric exercise. Time by treatment, p = 0.81. Values are mean (SE).

Sample size and statistical analysis

The sample size for this study was based on the difference in symptoms of muscle damage between arms using the same eccentric exercise protocol.[27] Based on the SD of the difference in strength loss between arms, it was estimated that, with a sample of 16 subjects, a 14% difference in strength loss could be detected between the placebo and cherry juice trials ($p<0.05$; power = 80%). The corresponding estimated effect sizes were 1.2 points for pain, 6 N for tenderness, and 6° for motion loss.

Changes in the symptoms of muscle damage (pain, tenderness, relaxed elbow angle, strength) between the cherry juice and placebo trials were assessed using treatment (cherry juice versus placebo) by time (baseline, 24, 48, 72, and 96 hours) repeated measures analyses of variance, with Bonferroni corrections on pairwise comparisons between placebo and treatment trials. Mean (SD) is reported in the text, and mean (SE) is shown in the figures.

RESULTS

Of the 16 subjects who started the protocol, two withdrew before completion. Both were students who left school at the end of the semester before completing the protocol. The remaining 14 subjects completed the study.

Isometric elbow flexion strength loss was significantly greater in the placebo trial than the cherry juice trial (treatment by time $p<0.0001$; fig 1). This effect was not different between test angles (angle by treatment by time p = 0.41). Strength loss (averaged across all three test angles) was 22 (12)% over the four days after the placebo trial but only 4 (15)% after the cherry juice trial ($p<0.0001$). Strength loss was not different between test angles (p = 0.31). In the placebo trial, strength loss was 24 (13)% at 130° of elbow flexion (short muscle length), 20 (16)% at 90°, and 20 (13)% at 30° (long muscle length). In the cherry juice trial, these values were 5 (19)%, 5 (18)%, and 2 (13)% respectively.

The development of pain in the elbow flexors after eccentric exercise was also significantly different in the placebo and cherry juice trials (treatment by time, p = 0.017; fig 2). Pain values (averaged across the four days) tended to be higher in the placebo trial (3.2 (1.1)) compared with the cherry juice trial (2.4 (0.7); p = 0.051). Pain peaked at 24 hours in the cherry juice trial (3.4 (1.2)) and subsequently declined, whereas pain continued to increase in the placebo trial to peak at 48 hours (4.5 (1.7)).

Loss of range of motion with the relaxed elbow angle measurement was not different between cherry juice and placebo trials (treatment by time, p = 0.85; fig 3). Mean motion loss for the four days after the cherry juice trial was 3.5 (6.0)° and 4.4 (5.6)° after the placebo trial.

Muscle tenderness was also not different between cherry juice and placebo trials (treatment by time, p = 0.81; fig 4). Mean tenderness for the four days after the cherry juice trial was 8.8 (9.7) N and 8.2 (10.1) N after the placebo trial (p = 0.84).

DISCUSSION

To our knowledge, this is the first study to examine the effect of consumption of cherries, or a cherry product, on symptoms of exercise induced muscle damage. Consumption of a cherry juice blend for three days before a bout of eccentric exercise and for the subsequent four days was shown to decrease some of the symptoms of muscle damage. Strength loss and pain were diminished in the cherry juice trial, but motion loss and muscle tenderness were unaffected.

A randomised crossover design was used because the variability in symptoms of muscle damage between limbs within a subject is typically less than the variability in symptoms between subjects.[27,28] The use of the contralateral arm rather than the same arm in the second trial (cherry juice or placebo depending on randomisation) avoided the impact of the repeated bout effect. Although it is well established that symptoms of muscle damage are diminished after a repeated bout of eccentric exercise, this protective effect does not cross over to the non-exercised limb.[28–30] Of the 16 subjects who began the study, nine started with the placebo trial and seven started with the cherry juice trial. However, the two subjects who left school before the end of the study started with the cherry juice trial. Therefore, only five of 14 subjects who completed the study started with the cherry juice trial. To verify that the apparent effect of cherry juice on diminished strength loss and pain was not due to a crossover protective effect, we reanalysed the data for the five subjects who started with the cherry juice trial. Despite the small sample size, the treatment by time interaction was significant for strength loss in this group of five subjects (p = 0.001). Strength loss was 3 (2)% for the cherry juice trial and 24 (3)% for the placebo trial. The treatment by time interaction was not significant for pain in these five subjects (p = 0.83). However, the pattern was similar to the whole group, with pain declining after 24 hours in the cherry juice trial and peaking at 48 hours in the placebo trial.

The lack of effect of cherry juice supplementation on muscle tenderness and relaxed elbow angle indicates that either these symptoms reflect different aspects of the injury response or the measurements were insensitive to real differences between cherry juice and placebo trials. Considering that muscle tenderness and pain typically follow the same time course, peaking two days after eccentric exercise of the elbow flexors,[25,26] it is unlikely that they reflect different aspects of injury. Tenderness and pain also peaked two days after exercise in the present study, and therefore the effect of cherry juice would have been expected to be apparent in both measures. The fact that the tenderness

CCAB/WSFC SAB Mtg (Mar 09) Page 54

measurement was only made at one site may have been a limiting factor. Tenderness was measured distally because peak tenderness has been shown to occur distally.[25 31] Although measurements at additional sites may have increased the ability to detect a difference between trials, it is important to note that tenderness values were very similar between the trials, indicating no effect of cherry juice supplementation. However, the tenderness measurement is a measure of the threshold of tenderness—that is, the force at which the subject first experiences discomfort. The measurement does not indicate the magnitude of tenderness for a fixed force application. Two studies have shown an effect of anti-inflammatory drugs on muscle tenderness.[7 8] The target muscle group was the quadriceps in both studies. In one study,[8] the subjects were asked to rate the soreness from 0 to 10 while a force transducer was pressed into the quadriceps at four different locations.[8] In the other study, the force required to elicit soreness was recorded from multiple sites on the quadriceps, and the product of soreness intensity (N) and area (number of 2 cm sites registering a soreness measurement below a ceiling of 50 N) was recorded for analysis.[7] The use of a larger muscle group and multiple sites probably improves the ability to detect treatment effects.

The relaxed elbow angle data reflect a similar lack of effect of cherry juice supplementation. Given that the average loss of motion was less than 5° in each trial and that the estimated effect size was 6°, this negative finding could be attributed to inadequate power to detect a real difference between trials. A more damaging eccentric exercise protocol or a larger sample size may be necessary to assess the effect of cherry juice supplementation on this marker of damage. Of note, only one of the six studies showing efficacy with anti-inflammatory drugs[5–10] used the elbow flexors,[10] and the relaxed elbow angle was not examined in that study.

Plasma or serum measures of myoglobin or creatine kinase activity are often used as markers of muscle damage, but were not used in this study. When using blood markers, it is important to control the activity levels of the subjects immediately before and during the study to ensure that other activities are not causing increases in these markers. Such restrictions were not thought to be necessary in this study because of the crossover design. In this study, subjects were screened for previous upper extremity strengthening exercise and instructed not to use their arms in strenuous activities during the study. However, they were not instructed to avoid exercising other body parts—for example, running—and therefore serum markers were not appropriate. Serum markers might be used in future studies where activity level is strictly controlled.

Although the results of this study indicate a protective effect of cherry juice, it is not possible to conclude that cherry juice supplementation prevented muscle damage, because only two of four indirect markers of damage showed an effect. However, there was clearly a preservation of muscle function attributable to the cherry juice. For the placebo trial, strength loss was 30% at 24 hours and still 12% at 96 hours after eccentric exercise. By contrast, in the cherry juice trial, strength loss was only 12% at 24 hours, and strength was actually 6% above baseline at 96 hours. Other studies have shown a treatment effect on isometric strength, but on a smaller magnitude. Loss of isometric knee extension strength was about 3% 24 hours after eccentric exercise in subjects taking ibuprofen four hours before exercise compared with about 13% for subjects taking a placebo.[7] Loss of isometric knee extension strength was about 15% 24 hours after eccentric exercise in subjects supplemented with vitamin C and E for 30 days compared with 27% in subjects taking a placebo.[19] By contrast, other studies have shown no effect of ibuprofen[11 13 14] or vitamin E and C[15–17 20] on strength loss.

What is already known on this topic

- Numerous antioxidant and anti-inflammatory agents have been identified in tart cherries, and consumption of cherries reduces circulating concentrations of inflammatory markers
- Many interventions have been studied for the prevention and treatment of exercise induced muscle damage but few have shown efficacy

What this study adds

- Consumption of cherry juice before and after eccentric exercise significantly reduced symptoms of muscle damage
- This is a practical intervention for alleviating the symptoms of muscle damage

Although it was not within the scope of this study to establish a specific mechanism of the preservation of strength, the hypothesis was that antioxidant and anti-inflammatory effects of cherry juice supplementation may lessen the damage response. The initial damage response of eccentric contractions is a mechanical disruption of myofibrils and injury to the cell membrane. When myofibrillar disruption is extensive, this triggers a local inflammatory response that leads to an exacerbation of damage.[32] Leukotrienes increase the vascular permeability, attracting neutrophils to the injury site, resulting in free radical production.[33] It is possible that the anti-inflammatory and/or the antioxidant effects of cherry juice mediated this secondary response and avoided the proliferation of myofibrillar disruption. This possibility could be examined in future work by measuring neutrophil and monocyte activation after eccentric exercise.

The apparent efficacy of this particular cherry juice in diminishing some of the symptoms of exercise induced muscle damage may be a function of the formulation of the drink. Consumption of about 45 cherries a day has been shown to reduce circulating concentrations of inflammatory markers in healthy men and women.[21] In the present study, each 12 oz bottle of cherry juice contained the equivalent of 50–60 cherries, and therefore subjects were consuming the equivalent of 100–120 cherries a day. In addition, the juice contained fresh cherries—that is, not from concentrate—and it is likely that this helped to preserve the phenolic compounds and anthocyanins. The concentrations of phenolic compounds and anthocyanins reported in the methods section can provide a reference for future studies examining the efficacy of similar supplements.

In conclusion, these data show efficacy for this cherry juice in decreasing some of the symptoms of exercise induced muscle damage. Most notably, strength loss averaged over the four days after eccentric exercise was 22% with the placebo but only 4% with the cherry juice. These results have important practical applications for athletes, as performance after damaging exercise bouts is primarily affected by strength loss and pain. In addition to being an efficacious treatment for minimising symptoms of exercise induced muscle damage, consumption of cherry juice is much more convenient than many of the treatments that have been presented in the literature.[33]

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Competing interests: the authors each have 2.5% equity in Cherrypharm Inc.

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COMMENTARY 1

The investigation offers originality and a significant contribution in the area of delayed onset muscle soreness and antioxidant/anti-inflammatory treatments. There are many studies in the literature on the use of more commonly known antioxidants such as vitamin C and vitamin E, with varying results. So this is both potentially promising and interesting.

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COMMENTARY 2

The question of what to do when muscles are sore and damaged has persisted for many years. An increasing number of studies have attempted to treat the symptoms of exercise induced muscle damage with strategies of growing complexity. Such treatments have included transcutaneous electrical stimulation, pulsed ultrasound, immobilisation, hyperbaric oxygen therapy, combined low intensity laser therapy/phototherapy, and compression sleeves, just to name a few. In many ways, these treatment strategies do not represent a practical or realistic option for either the competitive or recreational athlete. Other choices available for people with sore and damaged muscle are pharmacological treatments such as non-steroidal anti-inflammatory drugs; however, some may hesitate to ingest these pharmacological agents because of potential side effects or gastric discomfort. Thus the choices for relief from exercise induced muscle damage are limited. This study may have taken an important step toward providing a sensible and realistic treatment option for those suffering from sore and damaged muscles. The scientific question of how to treat the damaged muscle is an important one, and these researchers should be applauded for finding a potential treatment that is not only practical, but one that can be enjoyed!

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CCAB/WSFC SAB Mtg (Mar 09) Page 56

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Phytomedicine

Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries

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Summary

Anthocyanins from tart cherries, *Prunus cerasus* L. (Rosaceae) cv. Balaton™ and Montmorency; sweet cherries, *Prunus avium* L. (Rosaceae); bilberries, *Vaccinum myrtillus* L. (Ericaceae); blackberries, *Rubus* sp. (Rosaceae); blueberries var. Jersey, *Vaccinium corymbosum* L. (Ericaceae); cranberries var. Early Black, *Vaccinium macrocarpon* Ait. (Ericaceae); elderberries, *Sambucus canadensis* (Caprifoliaceae); raspberries, *Rubus idaeus* (Rosaceae); and strawberries var. Honeoye, *Fragaria* x *ananassa* Duch. (Rosaceae), were investigated for cyclooxygenase inhibitory and antioxidant activities. The presence and levels of cyanidin-3-glucosylrutinoside 1 and cyanidin-3-rutinoside 2 were determined in the fruits using HPLC. The antioxidant activity of anthocyanins from cherries was comparable to the commercial antioxidants, *tert*-butylhydroquinone, butylated hydroxytoluene and butylated hydroxyanisole, and superior to vitamin E, at a test concentration of 125 µg/ml. Anthocyanins from raspberries and sweet cherries demonstrated 45% and 47% cyclooxygenase-I and cyclooxygenase-II inhibitory activities, respectively, when assayed at 125 µg/ml. The cyclooxygenase inhibitory activities of anthocyanins from these fruits were comparable to those of ibuprofen and naproxen at 10 µM concentrations. Anthocyanins 1 and 2 are present in both cherries and raspberry. The yields of pure anthocyanins 1 and 2 in 100 g Balaton™ and Montmorency tart cherries, sweet cherries and raspberries were 21, 16.5; 11, 5; 4.95, 21; and 4.65, 13.5 mg, respectively. Fresh blackberries and strawberries contained only anthocyanin 2 in yields of 24 and 22.5 mg/100 g, respectively. Anthocyanins 1 and 2 were not found in bilberries, blueberries, cranberries or elderberries.

Key words: Anthocyanins, antioxidant, cyclooxygenase, *Prunus, Rubus, Fragaria, Vaccinum, Sambucus*

Introduction

Many compounds of plant origin are known to possess important phytoceutical or nutraceutical traits, such as their abilities to serve as cellular antioxidants by maintaining low levels of reactive oxygen intermediates or as anti-inflammatory agents by inhibiting prostaglandin synthesis. There is a growing interest in the utilization of phytoceuticals, in lieu of synthetic compounds, due to consumers increasingly negative perceptions of the safety of synthetic food additives (Namiki, 1990). We have investigated the bioactivities of the aglycone, cyanidin, and its glycosides from cherries and berries in our ongoing research on anthocyanins with antioxidant and cyclooxygenase inhibitory activities from fruits.

Anthocyanins are plant pigments responsible for the orange, red and blue colors of fruits, flowers, vegetables and other storage tissues in plants (Strack and Wray, 1993). Their presence is universally associated with attractive, colorful and flavorful fruits. Due to the uniqueness of anthocyanin profiles, these can be used

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to identify specific fruits and fruit products to determine product authenticity (Wrolstad et al., 1981). Anthocyanins have attracted much attention and are implicated with beneficial activities as food ingredients and as promoters of human health (Britt et al., 1998).

Previous research in our laboratory has shown that the tart cherry cultivars, *P. cerasus* L. cv. Balaton™ and Montmorency, are excellent sources of anthocyanins (Chandra et al., 1992, 1993; Wang et al., 1997). These compounds, cyanidin-3-glucosylrutinoside (**1**), cyanidin-3-rutinoside (**2**), cyanidin-3-glucoside (**3**) and their aglycone, cyanidin (**4**), have exhibited in vitro antioxidant and cyclooxygenase inhibitory activities comparable to those of commercial products (Wang et al., 1999). We now report the levels of these compounds in fresh fruits of sweet cherries and other consumed berries. In addition, we are reporting, for the first time, the COX-I and COX-II inhibitory activities of anthocyanin mixtures obtained from sweet cherries and some selected berries available in the market.

Materials and Methods

Fruit Samples

IQF (Individual Quick Frozen) tart cherries, *P. cerasus* L. cv. Balaton™ and Montmorency, and sweet cherries, *P. avium* L., were obtained from commercial growers (Traverse City, MI). Raspberries, *R. idaeus* L., and blackberries, *R.* sp., were purchased from Kroger and Meijer supermarkets (Okemos, MI), respectively. Blueberries var. Jersey, *V. corymbosum* L., strawberries var. Honeoye, *F.* x *ananassa* Duch., and cranberries var. Early Black, *V. macrocarpon* Ait., were obtained through the courtesy of Prof. Eric Hanson, Department of Horticulture, Michigan State University. Bilberries, *V. myrtillus* L., and elderberries, *S. canadensis*, were obtained as dried fruit powders from Assets Inc., NJ.

General Experimental Procedures

All ^1H NMR spectra were recorded on a Varian INOVA 300 MHz spectrometer. Chemical shifts were recorded in CD_3OD/DCl and are based in ρ (ppm) relative to CD_3OD (3.3 ppm). All solvents used were ACS reagent grade and were purchased from Sigma-Aldrich Chemical Co., Inc. Positive controls used in the antioxidant (ibuprofen and naproxen) and cyclooxygenase inhibitory (*tert*-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α-tocopherol) bioassays were purchased from Sigma Chemical Company.

Extraction of anthocyanins for HPLC analysis

Fresh fruits (20 g) were homogenized separately in water (10 ml) for 5 min in a Kinematica CH-6010 (Roxdale, Ontario, Canada) homogenizer and centrifuged (Model RC5C, Sorvall Instruments, Hoffman Estates, IL) at 10000 g for 20 min at 4 °C. The supernatant from each fruit sample was stored at –20 °C prior to analyses. Dried fruit powders were used for bilberries and elderberries, assuming a moisture content of 85% in fresh fruits.

HPLC conditions for analyses and quantification

All samples (20 µl injection volume) were filtered (0.22 µm) and analyzed on Capcellpak (Dychrom; Sunnyvale, CA) C-18 column (4.6 × 250 mm, 5 µm). The mobile phase, 4% aqueous H_3PO_4/CH_3CN (90:10 v/v), was used under isocratic conditions at a flow rate of 0.75 ml/min. Anthocyanins were detected at 520 nm using a PDA detector (Waters, Milford, MA). Quantification of anthocyanins **1** and **2** was accomplished using the Millenium 2010 chromatography manager Version 3.05.01 (Waters Corp.). Pure anthocyanins **1** and **2** were prepared earlier from Balaton™ tart cherries. Anthocyanins **1** and **2**, 1 mg each, were weighed separately and dissolved in 1 ml of the mobile phase (4% aqueous H_3PO_4/CH_3CN, 1:1). Two separate stock solutions were prepared for each anthocyanin and samples were then prepared by serial dilution of the individual stock solutions to afford 0.50, 0.25, 0.10, 0.05, 0.025 and 0.0125 mg/ml concentrations, respectively. Each sample was injected in duplicate and calibration curves were obtained by plotting the mean peak area percentages against concentration for each compound. Juice samples were analyzed in triplicate and the mean peak area percentages of anthocyanins were used to determine the quantities of anthocyanins **1** and **2** present.

Isolation of anthocyanins 1 and 2

Crude anthocyanins were prepared from Balaton™ tart cherries according to the method reported previously (Chandra et al., 1993; Wang et al., 1997) with the exception that XAD-16 resin (mesh size 20–60; Supelco, Bellefonte, PA) was used instead of XAD-2 resin (mesh size 20–50; Sigma Chemical Co., St. Louis, MO). The crude anthocyanin mixture (1 g) was dissolved in H_2O (2 ml) and fractionated using Medium Pressure Liquid Chromatography (MPLC) on a C-18 column (40 × 500 mm), eluting with increasing amounts of MeOH (0.1% HCl) in H_2O, starting with 30% MeOH to 100% MeOH. Five fractions, I: 250 ml, II: 250 ml, III: 300 ml, IV: 275 ml and V: 300 ml, were collected, evaporated *in vacuo* and then lyophilized. HPLC profiles revealed that fraction IV (25.7 mg) had a high content of anthocyanins **1** and **2**. Fraction IV was further purified by semi-prep. HPLC on a Capcellpak (Dychrom, Sunnyvale, CA) C-18 column (10 × 250 mm, 5 µm) to yield pure anthocyanins **1** and **2**, 7.8 mg and 9.3 mg, respectively. The mobile phase (4% aqueous H_3PO_4/MeOH, 70:30 v:v) was used under iso-

cratic conditions at a flow rate of 2 ml/min. The H_3PO_4 was removed from the fractions by adsorption on XAD-16 resin, washing with H_2O to a neutral pH, and elution with MeOH (0.1% HCl). The structures of anthocyanins **1** and **2** were confirmed using ^{1}H NMR spectroscopy and were identical to published data (Wang et al., 1997).

Cyanidin, the aglycone

The aglycone, cyanidin **4**, was prepared by acid hydrolysis of crude anthocyanin powder of Balaton™ tart cherries according to the method reported previously (Wang et al., 1997). The ^{1}H NMR spectrum was identical to that reported by Wang et al. (1997).

Purification of anthocyanins for bioassays

Lyophilized crude anthocyanin powders (50 mg) obtained from XAD-16 resin column were dissolved in H_2O (20 ml) and extracted with EtOAc (3 × 10 ml) to remove flavonoids and other polyphenols. The aqueous layer was concentrated and adsorbed on preconditioned (H_2O:MeOH, 1:1) C-18 Sep-Pak cartridges (Waters Associates, Milford, MA). The adsorbed pigments were subsequently washed with H_2O (5 × 1 ml), and then eluted with 4% aqueous H_3PO_4:MeOH, 1:1 (5 × 1 ml), to afford pure anthocyanins. The H_3PO_4 was removed by adsorption of anthocyanins on XAD-16 resin and washing with copious amounts of H_2O until a neutral pH was obtained, followed by elution with MeOH (0.1% HCl).

Cyclooxygenase-inhibitory assay

Cyclooxygenase I (COX-I) activity was determined using an enzyme preparation from ram seminal vesicles, purchased from Oxford Biomedical Research, Inc., Oxford, MI (ca. 0.46 mg protein/ml in 30 mM Tris buffer, pH 7). Cyclooxygenase II (COX-II) activity was conducted using a preparation from insect cell lysate diluted with Tris buffer (pH 7) to yield an approximate final concentration of 1.5 mg protein/ml (supplied by Dr. Dave Dewitt, Dept. of Biochemistry, MSU). COX assays were performed by monitoring the initial rate of O_2 uptake using an Instech micro oxygen chamber and electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH). Each assay mix contained 0.6 ml of 0.1 M Tris buffer (pH 7), 1 mmol phenol, 85 µg hemoglobin and 100 µmol arachidonic acid. Reaction was initiated by adding 5–25 µg of microsomal protein to test samples in a volume of 10–20 µl (Meade et al., 1993). Data were recorded using Quicklog for Windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA).

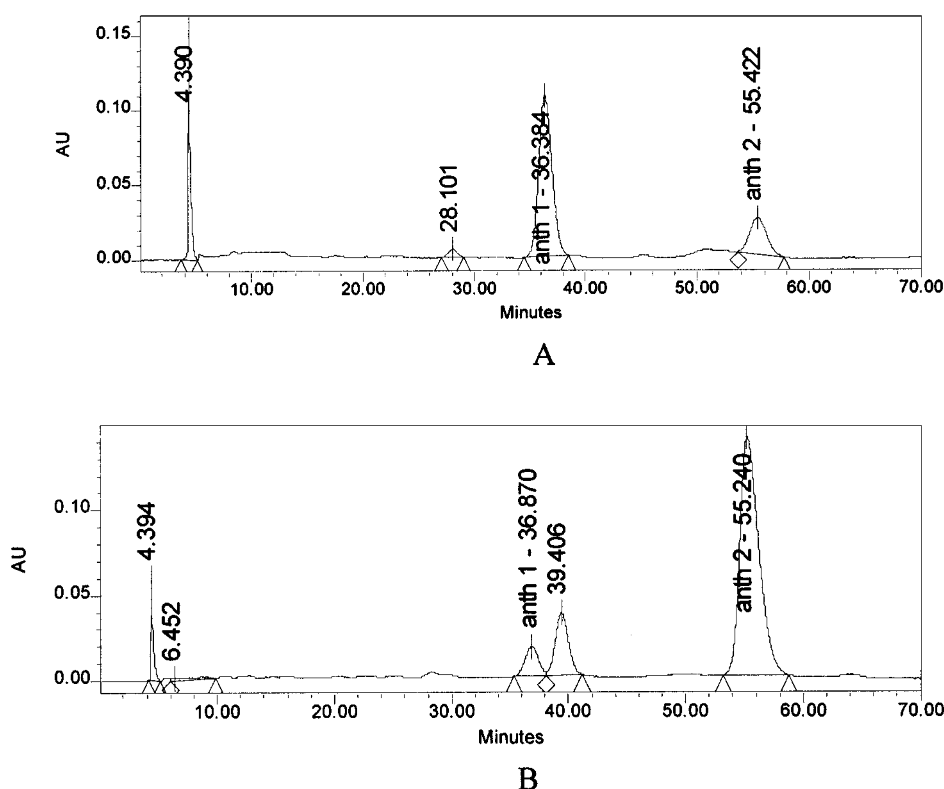

Fig. 1. HPLC chromatograms of anthocyanins from tart cherries cv. Balaton™ (A) and sweet cherries (B).

Antioxidant assay

Bioassays were conducted using analysis of model liposome oxidation using fluorescence spectroscopy. The lipid, 1-stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL), and fluorescent probe, 3-[p-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (Molecular Probes, Inc., Eugene, OR), were combined in DMF and dried *in vacuo* at RT. Large Unilamellar Vesicles (LUV's) were produced by resuspension of the lipid-probe mixture (0.15 M NaCl, 0.1 mM EDTA and 0.01 M MOPS maintained over Chelex resin) followed by ten freeze-thaw cycles in a dry ice-EtOH bath and extrusion (29 times) through a 100-nm pore size membrane (Avestin Inc., Ottawa, Canada). The final assay volume was 2 ml, consisting of 100 µl HEPES buffer (50 mM HEPES and 50 mM TRIS), 200 µl 1 M NaCl, 1.64 ml N_2 sparged water, 20 µl of test sample or DMSO (blank) and 20 µl aliquot of liposome suspension. Peroxidation was initiated by addition of 20 µl $FeCl_2 \cdot 4\ H_2O$ (0.5 mM) for positive controls (BHA, BHT, TBHQ and α-tocopherol/Vitamin E, all 10 µM) and test samples. Fluorescence was measured at 384 nm and monitored at 0, 1, 3 and every 3 min thereafter up to 21 min using a Turner Model 450 Digital Fluorometer (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity over time indicated the rate of peroxidation (Arora and Strasburg, 1997). Relative fluorescence (F_t/F_0) was calculated by dividing the fluorescence value at a given point (F_t) by that at t = 0 min (F_0).

Results

HPLC conditions utilizing the mobile phase of 4% aqueous H_3PO_4/CH_3CN under isocratic conditions gave excellent separation of the anthocyanins (Figure 1). Fresh Balaton™ and Montmorency tart cherry juices gave two major peaks at 36.38 and 55.42 min and corresponded to anthocyanins **1** and **2**, respectively. The HPLC profile of sweet cherry juice showed anthocyanin **2** at 55.24 min. Anthocyanin **3** was not detected in fresh tart and sweet cherry juices and was not quantified in this study. However, it was detected at 39.40 min in the enriched anthocyanin powder prepared from the fruits of sweet cherries under conditions identical to the case of tart cherries. The HPLC analysis and quantification of anthocyanin powder prepared from the fruits showed that sweet cherries contained predominantly anthocyanin **2** and a smaller amount of anthocyanin **1**, than tart cherries.

Anthocyanin concentrations in the cherries and berries were calculated from standard curves prepared using pure anthocyanins **1** and **2** isolated from fresh Balaton™ tart cherries. The yields of pure anthocyanins **1** and **2** in 100 g fresh Balaton™ and Montmorency tart cherries and sweet cherries were 21, 16.5; 11, 5 and 4.95, 21 mg, respectively.

The HPLC analysis of raspberries showed that it contained both anthocyanins **1** and **2**, eluting at 36.92 and 55.76 min, respectively, in addition to two other anthocyanins. The quantities of anthocyanins **1** and **2** in 100 g of fresh raspberries were 4.65 and 13.5 mg, respectively. The quantification of anthocyanins showed that fresh blackberries and strawberries var. Honeoye contained high levels of anthocyanin **2**, as compared to tart and sweet cherries, in yields of 24 and 22.5 mg/100 g, respectively. These fruits also contained anthocyanin **3**, detected as peaks at 39.22 and 39.44 min for blackberries and strawberries, respectively. Anthocyanins **1** and **2** were not detected in fresh blueberries var. Jersey and cranberries var. Early Black. Also, dried fruit powders of bilberries and elderberries did not reveal the presence of either anthocyanins **1** or **2**, which correlated with the report of Hong and Wrolstad (1990). The relative quantities of **1** and **2** in a 100 g sample of fresh fruits are shown in Figure 2.

Anthocyanin mixtures from the cherries and berries were tested for antioxidant activity by using an iron-catalyzed liposomal model and fluorescence spectroscopy to monitor the inhibition of lipid peroxidation (Arora and Strasburg, 1997). The anthocyanins prepared from the various fruits were tested at 125 µg/ml. Pure anthocyanins **1** and **2**, isolated from fresh Balaton™ tart cherries, were assayed at 10 µM whereas the aglycone, cyanidin **4**, was assayed at 5 µM. The results correlated closely with the report by Wang et al. (1999). The relative antioxidant activities of anthocyanin mixtures from the cherries and berries are shown in Figure 3.

The cyclooxygenase inhibitory assay was done using prostaglandin endoperoxide H synthase-I and -II (PGHS-I and -II) or cyclooxygenase (COX-I and -II) isozymes. These enzymes are used to measure the anti-inflammatory effects of natural products (Goda et al.,

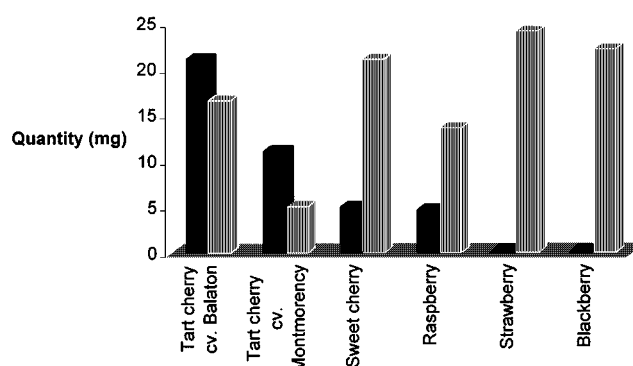

Fig. 2. Relative amounts of anthocyanin **1** (■) and anthocyanin **2** (▥) in 100 g fresh fruits.

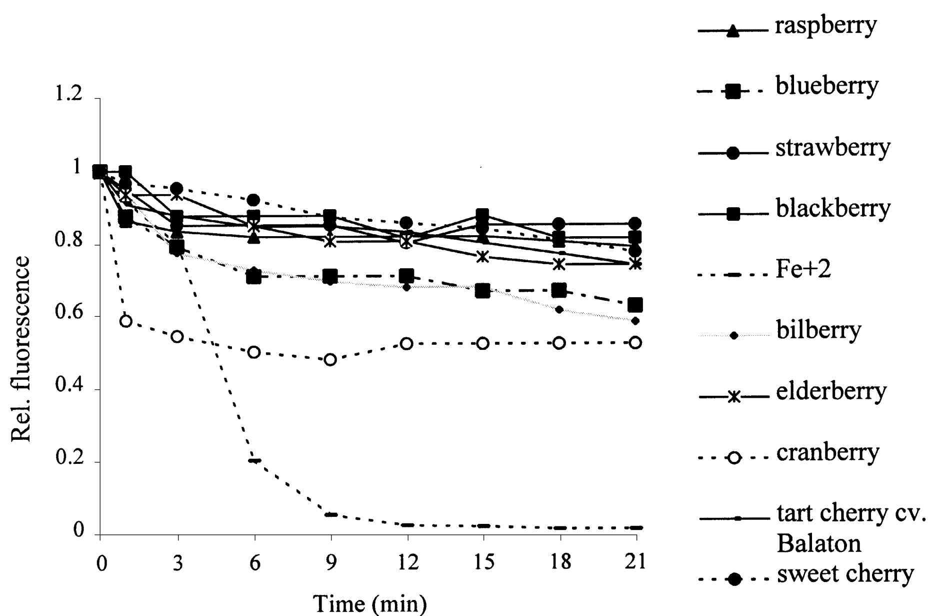

Fig. 3. Comparative antioxidant activities of anthocyanins from cherries and berries in a liposomal model system. Samples were tested at 125 µg/ml. In addition to solvent control DMSO, commercial antioxidants TBHQ, BHT, BHA and Vitamin E were also assayed at 10 µM concentrations (Wang et al., 1999).

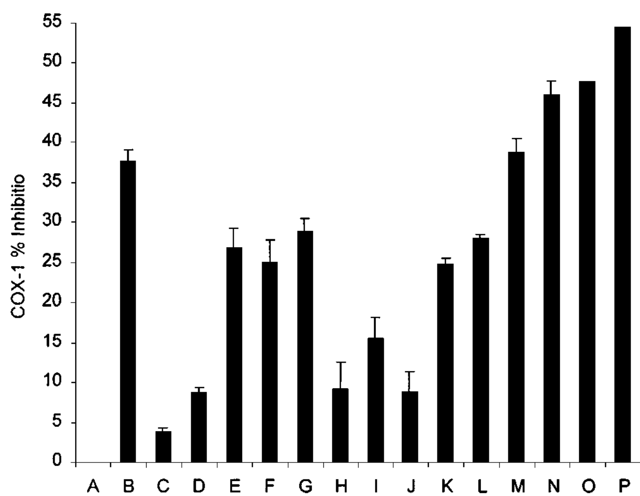

Fig. 4. COX-I enzyme inhibitory activity of anthocyanins (125 µg/ml) from cherries and berries. Cyanidin was tested at 5 µM and anthocyanins **1** and **2**, naproxen and ibuprofen were tested at 10 µM concentrations. The experiment was performed at pH 7. A – DMSO; B – cyanidin **4**; C – anthocyanin **1**; D – anthocyanin **2**; E – tart cherry cv. Balaton™; F – tart cherry cv. Montmorency; G – sweet cherry; H – blueberry var. Jersey; I – cranberry var. Early Black; J – bilberry; K – elderberry; L – strawberry var. Honeoye; M – blackberry; N – raspberry; O – naproxen; P – ibuprofen. Vertical bars represent the standard deviation of each data point (n = 2).

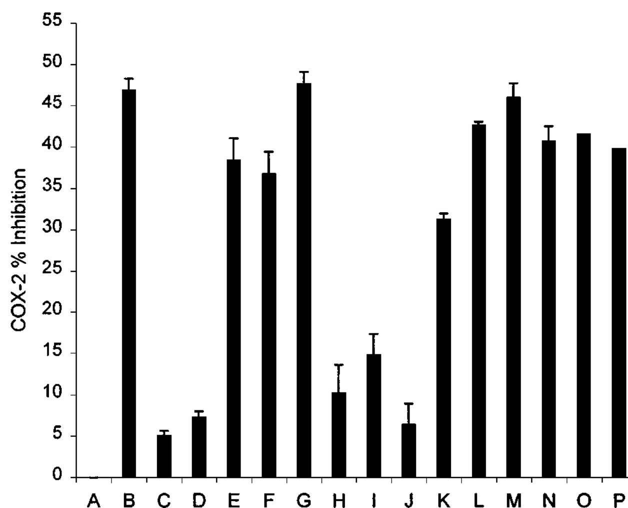

Fig. 5. Comparative COX-2 enzyme inhibition by anthocyanins (125 µg/ml) from various cherries and berries. Cyanidin was tested at 5 µM and anthocyanins **1** and **2**, naproxen and ibuprofen were tested at 10 µM concentrations. The experiment was performed at pH 7. A – DMSO; B – cyanidin **4**; C – anthocyanin **1**; D – anthocyanin **2**; E – tart cherry cv. Balaton™; F – tart cherry cv. Montmorency; G – sweet cherry; H – blueberry var. Jersey; I – cranberry var. Early Black; J – bilberry; K – elderberry; L – strawberry var. Honeoye; M – blackberry; N – raspberry; O – naproxen; P – ibuprofen. Vertical bars represent the standard deviation of each data point (n = 2).

1992) and the assay is based on the ability of the enzymes to convert arachidonic acid to prostaglandins, which evoke the physiological response of inflammation (Meade et al., 1993). We have assayed anthocyanin mixtures from the fruits at 125 µg/ml, pure anthocyanins **1** and **2** at 10 µm, and cyanidin **4** at 5 µM, concentrations. Positive controls used were ibuprofen and the nonsteroidal anti-inflammatory drug, naproxen, which showed 47.5 and 54.3% of COX-I and 39.8 and 41.3% of COX-II inhibitory activities, respectively, at 10 µM concentrations. The aglycone cyanidin **4** showed COX-I and -II inhibition activities comparable

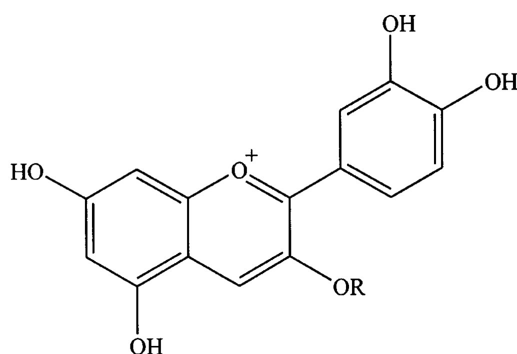

Fig. 6. Cyanidin-glucosylrutinose (**1**), cyanidin-rutinose (**2**) and cyanidin-glucoside (**3**), isolated from tart and sweet cherries, and cyanidin aglycone (**4**).
1 R = glucose, rhamnose, glucose; **2** R = glucose, rhamnose; **3** R = glucose; **4** R = H.

to ibuprofen and naproxen at 38.7 and 46.8%, respectively. Pure anthocyanins **1** and **2** displayed minimal COX activities (Figures 4 and 5).

Anthocyanins from raspberries and blackberries showed the best inhibition of the COX-I enzyme as compared to the other fruits, with 45.8 and 38.5% inhibitory activities, respectively. Anthocyanins from Balaton™ and Montmorency tart cherries, sweet cherries, strawberries and elderberries showed 26.6, 24.9, 28.8, 27.9 and 24.7% COX-I inhibition, respectively. Anthocyanins from cranberries, blueberries and bilberries showed lower COX-I activities than the other fruits with 15.5, 9.1 and 8.8% inhibition, respectively.

Anthocyanins from the cherries were active against the COX-II enzyme. Sweet cherries showed the best COX-II enzyme inhibition of all the fruits tested. Sweet cherries, Balaton™ and Montmorency tart cherries showed 47.4, 38.3 and 36.6% COX-II inhibition, respectively. For the berries, blackberry, strawberry, raspberry and elderberry showed activities of 45.7, 42.5, 40.5 and 31.2%, respectively. Cranberries, blueberries and bilberries showed 14.7, 10.1 and 6.4% inhibition, respectively, against COX-II.

Discussion

The anthocyanins, cyanidin-3-glucosylrutinoside (**1**), cyanidin-3-rutinoside (**2**) and cyanidin-3-glucoside (**3**), from the Michigan-grown tart cherry cultivars, Balaton™ and Montmorency, have been reported previously by our laboratory (Wang et al., 1997) (Figure 6). HPLC profiles revealed that sweet cherries and tart cherries contained the same anthocyanins. However, sweet cherries contained predominantly anthocyanin **2**. It has been reported previously that cyanidin **4** is the only aglycone present in varieties of sweet cherries, with cyanidin-3-rutinoside **2** and cyanidin-3-glucoside **3** as the major anthocyanins (Harbone and Hall, 1964). These data correlated with our results, although it should be noted that Lynn and Luh (1964) have reported the glycosides of both cyanidin and peonidin from sweet cherries. Through HPLC quantification, we have found that the concentrations of anthocyanins in Balaton™ and Montmorency tart cherries (IQF) were 375 and 160 mg per g fresh weight, respectively. However, we have observed that total anthocyanins in Balaton™ tart cherries can range from 200 to 400 mg/g fresh weight. Anthocyanin content of fruits should show variation due to nutritional, environmental and seasonal factors.

The HPLC profile of raspberry showed the presence of four major anthocyanins, which correlated with previous reports. It has been reported that anthocyanins **1**,, **2** and **3**, as well as cyanidin-3-sophoroside, are present in varieties of red raspberries (Harborne and Hall, 1964; Francis, 1972). This corresponded with anthocyanin **3** eluting at 39.38 min in the HPLC analysis of raspberries; hence; the peak at 28.24 min in the HPLC profile has been assigned to cyanidin-3-sophoroside. Torre and Barritt (1977) have reported that blackberries contain anthocyanins **2** and **3** as their minor and major pigments, respectively, which is in agreement with our findings. Our results for strawberries are very interesting and contrary to the report that the cultivated strawberries, *F. virginiana,* contain pelargonidin 3-glucoside as the major anthocyanin (Robinson and Smith, 1955). Although anthocyanin **3** has also been identified in wild strawberries (Sondheimer and Karash, 1956), it should be noted that strawberry var. Honeoye contained cyanidin-3-rutinoside **2** as its major anthocyanin.

Sapers et al. (1984) have separated as many as 16 anthocyanins by HPLC from highbush blueberries (*V. corymbosum* L.). These included anthocyanin **3** and other 3-glucosides of delphinidin, petunidin, peonidin and malvidin. Kalt et al. (1999a) have also investigated the anthocyanin content of both highbush and lowbush blueberries (*V. angustifolium* Aiton.) and have detected only anthocyanin **3** of the cyanidin glycosides found in tart and sweet cherries. The HPLC profile of fresh blueberries in our analysis showed that cyanidin-3-glucoside **3** eluted at 39.28 min.

It has been reported that the American cranberry (*V. macrocarpon* Ait.) is rich in the 3-galactosides and 3-arabinosides of peonidin and cyanidin (Zapsalis and Francis, 1965). Andersen (1989) has reported that fruits of the small cranberry, *V. oxycoccus* L., contained anthocyanin **3** and peonidin 3-glucoside as the main pigments. Of the cyanidin glycosides we have studied, only anthocyanin **3** was detected in cranberry var. Early Black.

Anthocyanins are known to have antioxidant activity and dietary antioxidants are believed to play a role in reducing the risks of various human degenerative dis-

eases. There are numerous reports on oxygen radical absorbing capacity (ORC) assay to measure antioxidant capacity of anthocyanins from fruits (Cao, et al., 1995; Kalt et al., 1999b; Wang and Lin, 2000). Our antioxidant assay is based on the reaction between a fluorescent probe and free radicals generated by a pro-oxidant such as Fe^{2+} (Arora and Strasburg, 1997).

The results indicated that antioxidant activity of the pure cyanidin glycosides increased with a decreasing number of sugar units; hence, anthocyanin **2** showed better activity than anthocyanin **1**. Similarly, the aglycone cyanidin **4** showed the best activity at much lower concentrations, and was comparable to the commercial antioxidants, butylated hydroxyanisole, butylated hydroxytoluene and *tert*-butyl hydroquinone, and superior to vitamin E (all 10 μM concentrations). The results for the anthocyanin mixtures from the fruits were the best for sweet cherries, although the other fruits also exhibited considerable antioxidant activities.

With the COX-I and -II enzymes, our results indicated that the aglycone cyanidin **4** showed superior inhibitory activity as compared to its glycosides. Cyclooxygenase inhibitory activities increased with a decreasing number of sugar residues attached to the cyanidin moiety; hence, anthocyanin **2** showed better activity than anthocyanin **1**. This correlated with observations by Wang et al. (1999). The mixture of anthocyanins obtained from the fruits showed considerable cyclooxygenase inhibitory activities as compared to the pure anthocyanins. The best COX-I and COX-II inhibitory activities were observed in the anthocyanins from raspberries and sweet cherries, respectively. Sweet cherries showed greater COX-II than COX-I inhibition and it should be noted that selective COX-II inhibitors are believed to be mainly responsible for anti-inflammatory activity (Masferrer et al., 1994).

The antioxidant and anti-inflammatory activities of the anthocyanins from these fruits suggest that their consumption would be beneficial to human health. Since these fruits contain a comparatively high level of anthocyanins, they may be included in a regular diet for alleviating arthritis- and gout-related pain.

Acknowledgments

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Comparison of Antioxidant Potency of Commonly Consumed Polyphenol-Rich Beverages in the United States

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A number of different beverage products claim to have antioxidant potency due to their perceived high content of polyphenols. Basic and applied research indicates that pomegranate juice (PJ), produced from the Wonderful variety of *Punica granatum* fruits, has strong antioxidant activity and related health benefits. Although consumers are familiar with the concept of free radicals and antioxidants, they are often misled by claims of superior antioxidant activity of different beverages, which are usually based only on testing of a limited spectrum of antioxidant activities. There is no available direct comparison of PJ's antioxidant activity to those of other widely available polyphenol-rich beverage products using a comprehensive variety of antioxidant tests. The present study applied (1) four tests of antioxidant potency [Trolox equivalent antioxidant capacity (TEAC), total oxygen radical absorbance capacity (ORAC), free radical scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP)]; (2) a test of antioxidant functionality, that is, inhibition of low-density lipoprotein (LDL) oxidation by peroxides and malondialdehyde methods; and (3) evaluation of the total polyphenol content [by gallic acid equivalents (GAEs)] of polyphenol-rich beverages in the marketplace. The beverages included several different brands as follows: apple juice (3), açaí juice (3), black cherry juice (3), blueberry juice (3), cranberry juice (3), Concord grape juice (3), orange juice (3), red wines (3), iced tea beverages (10) [black tea (3), green tea (4), white tea (3)], and a major PJ available in the U.S. market. An overall antioxidant potency composite index was calculated by assigning each test equal weight. PJ had the greatest antioxidant potency composite index among the beverages tested and was at least 20% greater than any of the other beverages tested. Antioxidant potency, ability to inhibit LDL oxidation, and total polyphenol content were consistent in classifying the antioxidant capacity of the polyphenol-rich beverages in the following order: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, açaí juice, cranberry juice > orange juice, iced tea beverages, apple juice. Although in vitro antioxidant potency does not prove in vivo biological activity, there is also consistent clinical evidence of antioxidant potency for the most potent beverages including both PJ and red wine.

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruits are popularly consumed in beverage forms such as pomegranate juice (PJ). Several studies have been conducted on a well-characterized PJ made from the Wonderful variety of *P. granatum* fruits (*1–6*). Basic and applied research in animals and humans indicates that this PJ has potent antioxidant activity, which has been linked to a diverse group of polyphenols including ellagitannins, gallotannins, ellagic acid, and flavonoids, such as anthocyanins (*1*). Whereas there are numerous phytochemicals consumed in our diet, polyphenols constitute the largest group and have attracted much attention due to their antioxidant properties (*7*). In fact, the potential health benefits of plant foods are commonly linked to their polyphenol content.

Currently, there are a number of commercial ready-to-drink (RTD) polyphenol-rich beverages, which base their marketing strategies on antioxidant potency. Apart from PJ, other popularly consumed RTD polyphenol-rich beverages that claim high antioxidant potency include red wine, berry fruit juices (e.g., blueberry, black cherry, Concord grape, cranberry, etc.), apple juice, bottled tea beverages, and, recently, the Amazonian palm

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Table 1. Antioxidant Potency of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States[a]

beverage	brand	DPPH (% inhibited)	ORAC (μmol of TE/mL)	FRAP (μmol of FE/mL)	TEAC (μmol/mL)
pomegranate juice	A	50.1 ± 1.9	25.0 ± 1.0	8.1 ± 0.3	41.6 ± 1.8
red wine	A	37.2 ± 1.8	26.7 ± 3.5	4.6 ± 0.1	19.8 ± 0.4
	B	31.7 ± 3.3	24.0 ± 2.0	3.8 ± 0.5	17.1 ± 0.9
	C	36.8 ± 1.4	26.5 ± 0.7	4.5 ± 0.2	19.2 ± 0.7
	av	35.2 ± 2.2	25.7 ± 2.1	4.3 ± 0.3	18.7 ± 0.7
Concord grape juice	A	27.4 ± 4.6	26.4 ± 1.9	5.0 ± 0.2	14.9 ± 0.6
	B	32.0 ± 8.4	30.5 ± 1.4	5.4 ± 1.1	21.7 ± 1.4
	C	25.1 ± 5.2	20.8 ± 2.2	4.4 ± 0.9	14.9 ± 1.1
	av	28.2 ± 6.1	25.9 ± 1.8	4.9 ± 0.8	17.2 ± 1.0
blueberry juice	A	31.3 ± 2.2	23.5 ± 2.6	4.7 ± 0.4	17.1 ± 0.5
	B	17.5 ± 0.8	23.9 ± 2.4	4.3 ± 0.1	14.7 ± 0.5
	C	13.0 ± 1.2	14.5 ± 3.8	3.6 ± 1.1	13.3 ± 0.6
	av	20.6 ± 1.4	20.6 ± 2.9	4.2 ± 0.5	15.0 ± 0.5
black cherry juice	A	10.9 ± 1.2	22.1 ± 2.0	3.3 ± 0.4	11.4 ± 0.5
	B	8.2 ± 1.5	22.1 ± 3.0	3.0 ± 0.6	11.6 ± 0.8
	C	14.8 ± 0.6	31.7 ± 4.9	3.8 ± 0.1	17.8 ± 0.2
	av	11.3 ± 1.1	25.3 ± 3.3	3.4 ± 0.4	13.6 ± 0.5
acai juice	A	21.3 ± 1.0	16.6 ± 0.6	4.3 ± 0.4	12.8 ± 0.4
	B	22.2 ± 1.9	22.9 ± 2.8	4.1 ± 0.6	16.2 ± 0.8
	C	14.8 ± 0.8	17.1 ± 1.2	3.5 ± 2.0	11.4 ± 0.6
	av	18.3 ± 1.2	19.5 ± 1.5	3.8 ± 1.0	12.8 ± 0.7
cranberry juice	A	19.2 ± 0.7	9.1 ± 1.0	2.2 ± 0.2	6.7 ± 0.3
	B	21.4 ± 0.6	21.5 ± 3.1	3.8 ± 0.8	14.8 ± 0.5
	C	17.1 ± 0.6	15.9 ± 2.1	2.2 ± 0.6	9.6 ± 0.4
	av	19.2 ± 0.6	15.4 ± 2.1	2.7 ± 0.5	10.4 ± 0.4
orange juice	A	12.9 ± 1.0	9.2 ± 0.3	1.5 ± 0.1	3.4 ± 0.4
	B	14.4 ± 0.6	6.1 ± 0.7	1.5 ± 0.5	4.4 ± 0.2
	C	10.9 ± 1.3	6.9 ± 0.4	1.5 ± 0.1	4.8 ± 0.3
	av	12.7 ± 1.0	7.4 ± 0.5	1.5 ± 0.2	4.2 ± 0.3
iced green tea	A	21.0 ± 0.5	5.8 ± 0.8	1.7 ± 0.1	6.8 ± 0.5
	B	24.3 ± 6.5	6.1 ± 3.9	1.9 ± 0.8	10.5 ± 0.5
	C	26.6 ± 2.3	6.0 ± 0.4	1.9 ± 0.4	7.5 ± 0.4
	D	17.5 ± 0.8	3.2 ± 2.4	1.5 ± 0.2	4.8 ± 0.2
	av	22.3 ± 2.6	5.3 ± 1.9	1.7 ± 0.4	7.4 ± 0.4
iced black tea	A	19.7 ± 0.9	5.9 ± 0.2	1.0 ± 0.2	5.3 ± 0.3
	B	11.1 ± 2.4	1.7 ± 0.4	0.5 ± 0.1	4.0 ± 0.2
	C	8.1 ± 1.7	1.8 ± 0.0	0.1 ± 0.0	1.5 ± 0.1
	av	13.0 ± 1.7	3.1 ± 0.2	0.5 ± 0.1	3.6 ± 0.2
iced white tea	A	21.6 ± 8.7	2.3 ± 0.3	1.1 ± 0.1	4.2 ± 0.4
	B	19.6 ± 0.9	4.8 ± 0.5	1.3 ± 0.2	6.9 ± 0.9
	C	5.1 ± 0.3	1.0 ± 0.1	0.2 ± 0.0	1.1 ± 0.5
	av	15.4 ± 3.3	2.7 ± 0.3	0.9 ± 0.1	4.1 ± 0.6
apple juice	A	15.4 ± 2.1	2.5 ± 0.3	1.4 ± 0.2	4.3 ± 0.3
	B	9.8 ± 2.8	5.7 ± 2.0	1.1 ± 1.2	3.6 ± 0.4
	C	10.2 ± 0.8	6.2 ± 0.8	1.0 ± 0.1	2.7 ± 0.3
	av	11.8 ± 1.9	4.8 ± 1.0	1.2 ± 0.5	3.6 ± 0.3

[a] TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant capacity; DPPH, free radical scavenging properties by diphenyl-1-picrylhydrazyl radical.

berry, *Euterpe oleraceae* Mart. (açaí), juice. However, to the best of our knowledge, data on the direct comparison of PJ's antioxidant activity to those of these widely available leading beverage products are unavailable. It is of great interest to the general public to know the antioxidant capacity of the beverages that they consume. However, it should be cautioned that because of the inherent complexity of food matrices, the use of one antioxidant capacity method to determine antioxidant potency is ineffective. This is because antioxidants respond to different reactive species in different tests, which is partially attributed to multiple reaction mechanisms and reaction phases (*8, 9*).

The aim of the current study was to compare the antioxidant potency of PJ to other leading brands of RTD polyphenol-rich beverages, available either nationally or regionally. Because no single antioxidant assay can accurately reflect the antioxidant potency of any beverage, we utilized four tests used to measure antioxidant potency [(1) Trolox equivalent antioxidant capacity (TEAC), (2) oxygen radical absorbing capacity (ORAC), (3) ferric reducing antioxidant power (FRAP), and (4) free radical scavenging properties by the diphenyl-1-picrylhydrazyl (DPPH) radical] and one test of antioxidant antioxidant functionality, namely, the inhibition of low-density (LDL) oxidation by

Table 2. Antioxidant Functionality of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States as a Measure of Ability To Inhibit Oxidation of Low-Density Lipoprotein (LDL) by the Peroxides and Malondialdehyde Methods

beverage	brand	inhibition of LDL oxidation (peroxides)	inhibition of LDL oxidation (malondialdehyde)
pomegranate juice	A	97.1 ± 0.0	97.2 ± 0.7
red wine	A	86.5 ± 5.6	69.7 ± 5.3
	B	70.2 ± 12.8	57.6 ± 12.5
	C	73.5 ± 7.3	56.6 ± 6.2
	av	76.7 ± 8.6	61.3 ± 8.0
grape juice	A	35.1 ± 10.5	31.1 ± 18.7
	B	46.5 ± 10.2	51.0 ± 18.4
	C	40.0 ± 6.0	26.7 ± 2.3
	av	40.5 ± 8.9	36.3 ± 13.1
blueberry juice	A	77.1 ± 10.6	59.1 ± 8.1
	B	42.9 ± 12.3	59.4 ± 6.1
	C	35.1 ± 5.9	17.8 ± 6.0
	av	51.7 ± 9.6	45.5 ± 6.8
black cherry juice	A	27.6 ± 3.1	10.0 ± 0.0
	B	25.1 ± 3.0	7.5 ± 1.6
	C	52.2 ± 2.1	82.9 ± 1.2
	av	35.0 ± 2.7	33.4 ± 0.9
acai juice	A	24.3 ± 0.9	14.5 ± 1.4
	B	29.2 ± 15.5	20.4 ± 6.7
	C	20.2 ± 1.2	14.2 ± 0.5
	av	21.7 ± 5.9	13.0 ± 2.8
cranberry juice	A	18.8 ± 2.4	21.2 ± 2.4
	B	58.2 ± 17.8	50.1 ± 11.6
	C	39.4 ± 10.0	45.6 ± 7.0
	av	38.8 ± 10.1	39.0 ± 7.0
orange juice	A	11.4 ± 7.6	9.8 ± 5.4
	B	16.9 ± 3.9	8.0 ± 2.6
	C	10.6 ± 0.6	5.3 ± 0.0
	av	12.9 ± 4.0	7.7 ± 2.7
apple juice	A	0.2 ± 0.3	−0.9 ± 2.7
	B	2.1 ± 3.2	0.6 ± 0.0
	C	2.7 ± 2.1	3.7 ± 2.7
	av	1.7 ± 1.9	1.1 ± 1.8
iced green tea	A	9.4 ± 5.1	11.4 ± 2.8
	B	12.0 ± 4.8	18.7 ± 0.3
	C	10.4 ± 0.7	3.9 ± 2.4
	D	4.9 ± 3.8	6.7 ± 3.1
	av	9.2 ± 3.6	10.2 ± 2.2
iced black tea	A	9.8 ± 1.9	13.7 ± 2.6
	B	6.9 ± 4.1	16.5 ± 3.3
	C	5.7 ± 1.8	17.9 ± 2.9
	av	7.4 ± 2.6	16.1 ± 2.9
iced white tea	A	9.5 ± 2.2	12.9 ± 1.3
	B	5.3 ± 1.7	9.5 ± 5.3
	C	1.5 ± 0.4	11.4 ± 2.0
	av	5.4 ± 1.4	11.3 ± 2.9

peroxides and malondialdehyde determinations. In addition, the beverages were also evaluated for polyphenol content as gallic acid equivalents (GAEs). The leading brands of beverages that PJ was compared to included apple juices (3), açaí juices (3), black cherry juices (3), blueberry juices (3), cranberry juices (3), Concord grape juices (3), orange juices (3), red wines (3), and iced tea beverages (10), consisting of black tea (3), green tea (4), and white tea (3).

MATERIALS AND METHODS

Ready-to-Drink Polyphenol-Enriched Beverages. The products used for the study are among the top brands in the selected beverage categories either nationally or regionally as follows: pomegranate juice (1), A, POM Wonderful 100% pomegranate (POM Wonderful LLC, Los Angeles, CA; 15MAY07Y0038, 16MAY07Y1804, 10MAY07Y0137); red wines (3), A, Merlot Beringer (Beringer Vineyards, Napa, CA; lot Founders Estate 2004-L11306B110, lot 3RD Century 2004-1101611, lot Founders Estate 2004-1 1130 6B 117), B, Zinfandel Robert Mondavi (Robert Mondavi, Woodbridge, CA, lot Private Selection 2005-LW34760602, lot Private Selection 2005-L2020106, lot Private Selection 2005-LW3450602), C, Cabernet Sauvignon Turning Leaf (Turning Leaf Vineyards, Modesto, CA, lot 2005-LB300107HE, lot 2005-LB171006DE, lot 2005-LB060206HH); Concord grape juices (3), A, RW Knudsen-Just Concord (Knudsen & Sons Inc., Chico, CA; lot NOV 29 2008 6 333 003, lot DEC 08 2007 5 342 003, lot DEC 09 2007 5 343 003), B, Lakewood-Pure Concord Grape (Lakewood, Miami, FL; lot 146S FEB232009, lot OCT252008, 4/10/2007, lot MAR012008), C, Welch's Grape Juice (Welch's, Concord, MA; lot DEC-11-07 6NL11L1451, lot 7N17A1048 JAN-17-08, lot PL 22 B11 FEB-23-08); blueberry juices (3), A, RW Knudsen-Just Blueberry (Knudsen & Sons Inc.; FEB 14 2009 7 045 003, NOV 16 2008 6 320 003, SEP 20 2008 6 263 003), B, Trader Joe's Just Blueberry (distributed by Trader Joe's, Monrovia, CA; JAN 23 2008 7 023 003, JAN 10 2008 7 010 003, JUL 13 2007 6 194 003), C, Wyman's Wild Blueberry (Jasper Wyman & Son, Milbridge, ME; 8036 CA1 0903 CT 803, C136CA 1 0534 CT 803, 1267CA 0214 1 CT 803); black cherry juices (3), A, RW Knudsen-Just Black Cherry (Knudsen & Sons Inc.; JAN 18 2009 6 199 003, DEC 6 2008, DEC 14 2008 6 165 003), B, Lakewood-Pure Black Cherry (Lakewood; regular, red cap with vitamin C, 3/23/2007, organic, 141 VADA, organic, gold cap), C, Trader Joe's Just Cherry (distributed by Trader Joe's; JAN 23 2008 7 023 003, 3/23/2007, DEC 13 2007 6 347 003, DEC 21 2007 6 355 003); acai juices (3), A, Bolthouse Bom Dia Acai-Mangosteen (Bolthouse Juice Products LLC, Bakersfield, CA; lot 061107, lot 051107, lot 062607), B, Bossa Nova Acai Original (Bossa Nova Beverage Group Inc., Los Angeles, CA; lot 09 16 07, lot 10 10 07, lot 10 09 07), C, Sambazon Mango Uprising (Sambazon, San Clemente, CA; lot ASA07029 APR 2007, lot 0610T HA16PTK13, 4/07/2007, lot ASA07073 12 JUN 2007); cranberry juices (3), A, Northland 100% Juice Cranberry (distributed by Northland Products LLC, Port Washington, NY; lot 02/28/08, lot 02/13/08, lot 03/06/08), B, RW Knudsen-Just Cranberry (Knudsen & Sons Inc.; lot NOV 21 2008 6 325 003, lot JAN 11 2009 7 011 003, lot FEB 07 2009 7 038 003), C, Ocean Spray-Pure Cranberry (Ocean Spray Cranberries Inc., Lakeville-Middleboro, MA; lot MAY 28 07 CT841 CP1); orange juices (3), A, Florida's Natural Orange Juice (Florida's Natural Growers, division of Citrus World Inc., Lake Wales, FL; lot JUN 05 07, lot JUN 08 07, lot MAY 15 07), B, Tropicana Pure Premium Orange Juice (Tropicana Products Inc., Bradenton, FL; lot MAY 15 07 48NK0713, lot JUN 29 07 48NK0647, lot JUN 15 07 46NL1446), C, Minute Maid Premium Orange Juice (Minute Maid, produced for Coca-Cola Co., Atlanta, GA; lot APR 30 07 DN, lot MMOJ 02810 APR 30 07 1030 DN1CR, lot MAY 7 07 DN); iced green tea (4), A, Tazo Iced Green Tea (bottled for Tazo, Portland, OR; lot BB08NOV2007 L08NOV20060315, lot BB08JAN2008 L08JAN20070313, lot BB18OCT2007 L18OCT20060312), B, Honest Just Green Tea (Honest Tea Inc., Bethesda, MD; lot H06314, lot H06122), C, Lipton Original Green Tea-Honey (manufactured for Pepsi-Lipton Tea Partnership, Purchase, NY; lot MAY0707V 2237 YY 10306 3, lot AUG2707 0742 YY 02227 3, lot SEP2407 59745 2336 YY 03247 3), D, Snapple Green Tea-Mango (distributed for Snapple Beverage Corp., Rye Brook, NY; lot FC292 LPG 111506 D, lot 6200 12 1228, lot 6300 12 1230); iced black tea (3), A, Tazo Iced Tea with Lemon (bottled for Tazo; lot BB08MAY2007, L08MAY20060316, lot BB29JUN2007 L29JUN20060319, lot BB13NOV2007 L13NOV20060317), B, Lipton Original Iced Tea (manufactured for Pepsi-Lipton Tea Partnership; lot OCT2907 0926 YY 01257 3, lot NOV2607 0759 YY 02217 3, lot SEP 10 07 YY 120863), C, Nestea Sweetened Lemon Flavored Iced Tea (Nestle USA or Beverage Partners Worldwide, lot OCT1507TRB 08103, lot NOV0507TRC 19133, lot

Table 3. Phenolic Content, as Gallic Acid Equivalents (GAEs), of Commonly Consumed Beverages and Their Primary Antioxidant Phytochemicals As Reported in the Literature

beverage	GAEs (mg/mL)	primary antioxidant phytochemicals
pomegranate juice	3.8 ± 0.2	ellagitannins and anthocyanins (*30*)
red wine	3.5 ± 0.1	proanthocyanidins, anthocyanins, catechins, and flavonoids (*31*)
Concord grape juice	2.6 ± 0.4	proanthocyanidins, anthocyanins, catechins, flavonoids, and vitamin C added (*31*−*33*)
blueberry juice	2.3 ± 0.4	proanthocyanidins, catechins (*34*), anthocyanins (*35*), and other phenolic acids (*36*)
black cherry juice	2.1 ± 0.1	anthocyanins, flavonoids, flavan-3-ols, and other phenolic compounds (*37*)
acai juice	2.1 ± 0.1	proanthocyanidins, flavonoids, and anthocyanins (*38*)
cranberry juice	1.7 ± 0.2	proanthocyanidins, flavonoids, and anthocyanins (*39*)
orange juice	0.7 ± 0.1	flavonoids, phenolic acids, and vitamin C (*40*)
apple juice	0.4 ± 0.1	polyphenolic acid, flavonoids, proanthocyanidins (*41, 42*)
iced green tea	0.8 ± 0.1	catechins and phenolic acids (*43*)
iced black tea	0.4 ± 0.0	theaflavones, catechins, and phenolic acids (*44*)
iced white tea	0.9 ± 0.0	catechins and phenolic acids (*45*)

SEP1707TRC 11033); iced white tea (3), A, Snapple White Tea Nectarine (distributed for Snapple Beverage Corp.; lot LLP11C7 17022, lot FC292 LPG 111706 004421, lot FC292 LPG 121806), B, Honest Mango White Tea (Honest Tea Inc.; lot H06361, lot H0633,), C, Inko's White Tea Original (bottled for Inko's White Iced Tea, New York, NY; lot CT90 A03308, lot CT90 A103208); apple juices (3), A, Dole Apple Juice (manufactured for Pepsico, Purchase, NY; lot AUG 06 07 JL 12066 2, lot NOV 05 07 JL 03057 2, lot SEP 10 07 JL 01097), B, Tree Top Apple Juice (Tree Top Inc., Selah, WA; 3067C LN1 03/05/08, 02/04/08 2047X 0503, 10/20/08 A206A), C, Mott's Apple Juice (manufactured for Motts LLP, Rye Brook, NY; lot 020807TA APR 08 08, lot 020707TA APR 07 08, lot 021207TA APR 12 08). All fruit juices, wines, and iced tea beverages were analyzed in late March or early April prior to their expiration dates as stated on their packages. All beverages were kept at storage conditions as specified on their labels prior to analyses. Chain-of-custody forms to verify unopened products within the same dates codes were generated for all test samples and are archived in our laboratory.

Determination of Total Polyphenols. Total polyphenols were determined spectrophotometrically according to the method of Singleton (*10*), modified for small volumes (*11*), and are reported as gallic acid equivalents (GAEs). Gallic acid stock solution was prepared in ethanol at a concentration of 1 mM.

Antioxidant Assays. *Trolox Equivalent Antioxidative Capacity.* The assay was performed as previously reported (*12*). Briefly, 2′,2′-azinobis(3-ethylbenzothiazline-6-sulfonic acid) diammonium salt (ABTS) radical cations were prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of $ABTS^{\bullet+}$ (20 mL using a 75 mM Na/K buffer of pH 7). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was used as an antioxidant standard. A standard calibration curve was constructed for Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 μM concentrations. Samples were diluted appropriately according to antioxidant activity in Na/K buffer pH 7. Diluted samples were mixed with 200 μL of $ABTS^{\bullet+}$ radical cation solution in 96-well plates, and absorbance was read (at 750 nm) after 5 min in a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA). Samples were assayed in six replicates. TEAC values were calculated from the Trolox standard curve and expressed as Trolox equivalents (in millimolar).

Total Oxygen Radical Absorbance Capacity. The ORAC assays were performed at Covance Analytical Laboratories, Inc. (Madison, WI), and were conducted as previously described (*12*). Briefly, a mixture of 0.125 mL of fluorescein (0.16 μM) was used as the target of free radical attack, and 0.250 mL of 2,2′-azobis(amidinopropane) dihydrochloride (AAPH) (147 mM) was used as a peroxyl radical generator at 37 °C combined with 0.250 mL of each sample extract. Trolox standards ranged from 5 to 40 μM. The decrease in fluorescence of fluorescein was determined by collecting readings at excitation of 535 nm and emission of 595 nm every minute for 45 min in a Molecular Devices SpectraMax M2 plate reader. The ORAC value was evaluated as the area under curve (AUC) and calculated by taking into account the Trolox reading using the following equation: $(AUC_{sample} - AUC_{buffer})/(AUC_{Trolox} - AUC_{buffer}) \times$ dilution factor of sample $\times$ initial Trolox concentration (mM). For each sample, four serial dilutions in phosphate buffer (pH 7.4) were measured.

Free Radical Scavenging Capacity. The free radical scavenging capacity was analyzed by the DPPH assay (*13, 14*). DPPH is a radical-generating substance that is widely used to monitor the free radical scavenging abilities (the ability of a compound to donate an electron) of various antioxidants. The DPPH radical has a deep violet color due to its impaired electron, and radical scavenging can be followed spectrophotometrically by the loss of absorbance at 517 nm, as the pale yellow nonradical form is produced. Aliquots from the analyzed compounds were mixed with 1 mL of 0.1 mM DPPH/L in ethanol and the change in optical density at 517 nm was continuously monitored using a Molecular Devices SpectraMax M2 plate reader.

Ferric Reducing Antioxidant Capacity Assay. The FRAP assays were performed using established standardized methods previously described at Covance Analytical Laboratories, Inc. (*15*). Reaction mixtures were prepared by combining 10 mM 2,4,6-tri[2-pyridyl-*s*-triazine] (TPTZ), 20 mM ferric chloride, and 300 mM (pH 3.6) sodium acetate buffer in a 1:1:10 ratio. Ferrous sulfate standards ranged from 100 to 1000 μM. A 0.3 mL portion of reaction solution was heated at 37 °C for 10 min, and then 0.020 mL of aqueous sample extracts was added. Sample absorbance was then read at 593nm in a Molecular Devices SpectraMax M2 plate reader and using linear regression results are in terms of millimolar ferric ions converted to the ferrous form per milliliter.

Inhibition of Low-Density Lipoprotein Oxidation. LDL was isolated from plasma derived from healthy normolipidemic volunteers, by discontinuous density gradient ultracentrifugation (*16*). The LDL was washed at d = 1.063 g/mL and dialyzed against 150 mmol/L NaCl and 1 mmol/L Na_2EDTA (pH 7.4) at 4 °C. The LDL was then sterilized by filtration (0.45 μM), kept under nitrogen in the dark at 4 °C, and used within 2 weeks. LDL (100 μg of protein/mL) was incubated for 10 min at room temperature with the beverages. Then, 5 μmol/L of $CuSO_4$ was added, and the tubes were incubated for 2 h at 37 °C. Cu^{2+}-induced oxidation was terminated by the addition of butylated hydroxytoluene (BHT, 10 μM) and an immediate storage at 4 °C. At the end of the incubation, the extent of LDL oxidation was determined by measuring the generated amount of lipid peroxides and also by the thiobarbituric acid reactive substances (TBARS) assay at 532 nm, using malondialdehyde (MDA) for the standard curve (*17, 18*).

Statistical Analysis. Antioxidant capacity values were determined in six replicates for each sample tested, and the mean values ± standard deviation (SD) are reported. An overall antioxidant potency composite index was determined by assigning all assays an equal weight, assigning an index value of 100 to the best score for each test, and then calculating an index score for all other samples within the test as follows: antioxidant index score = [(sample score/best score) × 100]; the average of all seven tests for each beverage was then taken for the antioxidant potency composite index. All assays were given equal weight, and an overall mean index value was calculated on a normalized basis for each beverage. A simple rank order was reported, and where the values were close to each other, an equal rank was assigned.

Table 4. Antioxidant Potency Composite Index of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States[a]

beverage	brand	DPPH index	ORAC index	FRAP index	TEAC index	antioxidant potency composite index[b]
pomegranate juice	A	100.0	78.9	100.0	100.0	95.8
red wine	A	74.3	84.2	56.8	47.6	72.0
	B	63.3	75.7	46.9	41.1	63.3
	C	73.5	83.6	55.6	46.2	69.6
	av	70.3	81.1	53.1	45.0	68.3
Concord grape juice	A	54.7	83.3	61.7	35.8	57.6
	B	63.9	96.2	66.7	52.2	70.0
	C	50.1	65.6	54.3	35.8	57.5
	av	56.3	81.7	60.5	41.3	61.7
blueberry juice	A	62.5	74.1	58.0	41.1	60.8
	B	34.9	75.4	53.1	35.3	52.4
	C	25.9	45.7	44.4	32.0	40.7
	av	41.1	65.0	51.9	36.1	50.9
black cherry juice	A	21.8	69.7	40.7	27.4	42.4
	B	16.4	69.7	37.0	27.9	40.2
	C	29.5	100.0	46.9	42.8	57.0
	av	22.6	79.8	42.0	32.7	46.5
acai juice	A	42.5	52.4	53.1	30.8	46.8
	B	44.3	72.2	50.6	38.9	54.4
	C	29.5	53.9	43.2	27.4	44.0
	av	36.5	61.5	46.9	30.8	46.2
cranberry juice	A	38.3	28.7	27.2	16.1	27.3
	B	42.7	67.8	46.9	35.6	52.8
	C	34.1	50.2	27.2	23.1	33.7
	av	38.5	48.9	33.3	24.8	38.0
orange juice	A	25.7	29.0	18.5	8.2	20.0
	B	28.7	19.2	18.5	10.6	19.1
	C	21.8	21.8	18.5	11.5	17.9
	av	25.3	23.3	18.5	10.1	19.1
apple juice	A	30.7	7.9	17.5	10.3	15.2
	B	19.5	17.9	14.0	8.7	14.1
	C	20.3	19.5	12.3	6.5	13.8
	av	23.6	15.1	14.8	8.7	14.6
iced green tea	A	41.9	18.3	21.0	16.3	23.2
	B	48.5	19.2	23.5	25.2	27.5
	C	53.1	18.9	23.5	18.0	29.0
	D	34.9	10.1	18.5	11.5	17.1
	av	44.5	16.7	21.0	17.8	24.2
iced black tea	A	39.3	18.6	12.3	12.7	19.8
	B	22.2	5.4	6.2	9.6	10.8
	C	16.2	5.7	1.2	3.6	6.4
	av	25.9	9.8	6.2	8.7	12.2
iced white tea	A	43.1	7.3	13.6	10.1	23.8
	B	39.1	15.1	16.0	16.6	21.1
	C	10.2	3.2	2.5	2.6	4.7
	av	30.7	8.5	11.1	9.9	16.8

[a] TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant capacity; DPPH, free radical scavenging properties by diphenyl-1-picrylhydrazyl radical. [b] Antioxidant index score = [(sample score/best score) × 100], averaged for all seven tests for each beverage for the antioxidant potency composite index.

RESULTS

Table 1 shows the antioxidative potency (by TEAC, DPPH, ORAC, and FRAP assays) and **Table 2** shows the antioxidant functionality (by inhibition of LDL oxidation) of the leading types of RTD polyphenol-rich antioxidant beverage categories sold in the United States. **Table 3** shows the phenolic content, as GAEs, of the commonly consumed beverages, and their primary antioxidant phytochemicals as reported in the literature. **Table 4** shows the antioxidant potency composite index determined for the beverages based on ranking of all four antioxidant assays, TEAC, DPPH, ORAC, and FRAP.

Generally, it is known that total polyphenols are highly correlated with antioxidant activity, and the bioavailability of polyphenols has been reported (*19*). As shown in **Figure 1**, as a group, the ordering of the average amounts of total phenolics for the beverages was as follows: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice.

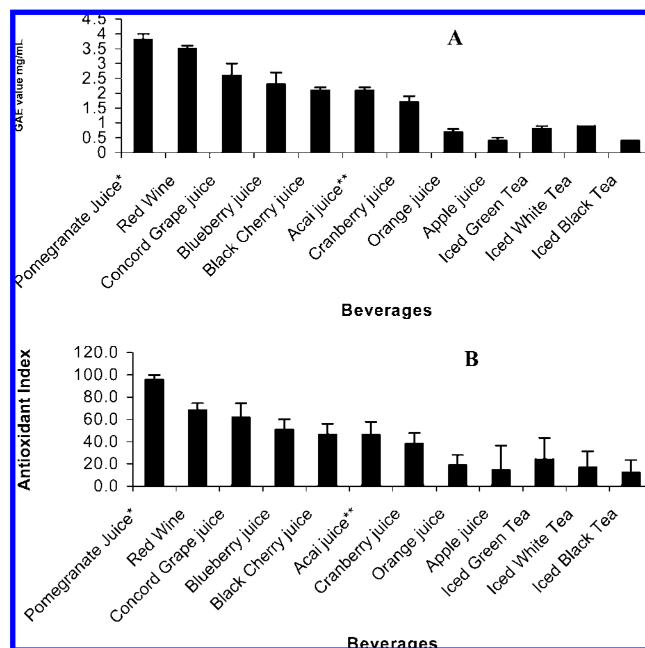

Figure 1. (**A**) Total phenolics in ready-to-drink polyphenol-rich antioxidant beverages as gallic acid equivalents (GAEs) and (**B**) antioxidant potency composite index of ready-to-drink polyphenol-rich antioxidant beverages calculated as a percentage of average antioxidant activities compared to the highest one in each assay and sum of the individual index divided by the number tested (four assays in total: DPPH, ORAC, TEAC, and FRAP). Each beverage had three different batches, and each sample was analyzed in triplicate ($n = 3$). *, POM Wonderful 100% pomegranate juice. **, the acai juices in **Figure 1** and **Tables 1** and **2** did not include Mona Vie, the premier acai blend, because it is a blend of acai and 18 other fruit juices. The Mona Vie data show the polyphenol and antioxidant index to be in the same range as for the acai juices reported or in the midrange for all beverages analyzed in this study (unpublished data).

The order of antioxidant potency showed the same trend as the total phenolic content in the beverages as follows: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice (see **Figure 1**). Similarly, as a test of antioxidant functionality, the LDL susceptibility to oxidation values was highly correlated with both total polyphenol content and antioxidant capacity assays as follows: PJ > red wine > blueberry juice > Concord grape juice, cranberry juice, black cherry juice > acai juice, orange juice, iced tea beverages, apple juice. However, the most widely marketed method of determining antioxidant capacity is the ORAC method, and this method gave a rank order differen from the above methods as follows: Concord grape juice, red wine, PJ, black cherry juice > blueberry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice. Nevertheless, when all of the methods were combined into a single index of antioxidant activity (**Table 4**), the rank order was as shown in **Figure 1**: PJ > red wine > grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > iced tea beverages, orange juice, apple juice. PJ's overall antioxidant index was at least 20% higher than any of the other beverages tested, thereby displaying the most complete free radical neutralizing range/bandwidth.

DISCUSSION

Being a polyphenol-rich food with health benefits has become a more common element in food marketing. The public is highly aware of the term "antioxidant", which has been defined by the Institute of Medicine of the National Academy of Sciences as follows: "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic function in humans." Therefore, the marketing of many so-called "superfoods" is commonly based on their antioxidant potential. In fact, a number of antioxidant foods claim to have superior antioxidant activity with health benefits based on in vitro antioxidant assays, and a limited number also have clinical evidence demonstrating effects on physiological function that can be related to oxidant protection.

In the present study, among the most popular national brands of polyphenol-rich antioxidant beverages including 100% fruit juices, iced tea beverages, and red wine, PJ had the most potent antioxidant capacity followed by red wine and grape juice (see **Figure 1** and **Table 4**). The order of antioxidant capacity was very consistent across the different methods with the exception of the ORAC method. The ORAC method is the most widely recognized assay used by food manufacturers but has significant internal variability. Using the ORAC assay, the antioxidant activity is determined as area under the curve of a 60 min measurement of the protection from oxidation by free radicals (AAPH) generated in a temperature-dependent reaction. On the basis of technical issues related to temperature gradients across the plate in commonly used plate readers, this assay can have significant internal variabilities.

The ORAC method has been applied extensively to evaluate the antioxidant capacity of a large variety of foods (*20, 21*), and many supplement and functional food companies compare their products, including juices, favorably to fruits and vegetables using the ORAC results from those studies. In fact, Prior et al. also evaluated some of the fruit juices used in our study, and there is a good agreement with the ranking (*22*). However, our laboratory has demonstrated that temperature variation in the plate readers used in this assay leads to increased variability of this method (unpublished data). Although this technical issue does not pertain to the end point determinations used in the TEAC, FRAP, and DPPH assays or to the assays of LDL oxidation, we chose to include the data from the ORAC assay in our overall determination of an antioxidant index for fruit juice beverages. Therefore, in our view, it is important to run multiple antioxidant methods rather than just the ORAC method to get a better estimate of antioxidant capacity and to substantiate in vitro results with clinical studies. Furthermore, because in vitro results are not necessarily translated into in vivo effects, issues such as the bioavailability and metabolism of phenolic compounds should be taken into account in the overall evaluation of the impact of "phenolic/antioxidant-rich" foods on human health.

Multiple assays with different sensitivities and specificities for antioxidant activity are being used separately to justify health claims. At the 2007 meeting of the Institute of Food Technologists, a number of new polyphenol-rich fruits were being identified as "superfruits" including acai, mangosteen, noni, sea buckthorn, and Chinese wolfberry (goji). Consumers have a difficult time distinguishing among the various antioxidant claims for widely available antioxidant beverages even without considering these newer entries to the marketplace. Therefore, the present study was significant in comparing the most commonly available national brands of RTD beverages for antioxidant activity using the most well-known laboratory methods for determining antioxidant capacity.

PJ had the highest antioxidant capacity and the most complete antioxidant coverage in vitro. In addition, there is extensive evidence of physiological activity of this juice in humans with regard to intima media thickness (*5*), cardiac blood flow (*23*),

prostate cancer progression (*2*), erectile dysfunction (*24*), and type 2 diabetes mellitus (*25*). All of these can arguably be related to protection from oxygen radicals or closely related anti-inflammatory effects of antioxidant phytochemicals (*26*). The bioavailability of PJ polyphenols and active metabolites has also been extensively studied to support the translation of this in vitro research into in vivo bioactivity (*1, 27*).

This study applied in vitro antioxidant capacity testing to reflect the multiple antioxidant capacity tests using different reagents to provide a more complete profile of antioxidant capacity. The present research demonstrates that although a number of popular beverages have evidence of antioxidant activity in vitro, there are clear differences in antioxidant potency. Some beverages with lower potency would need to be consumed in much larger amounts to equal the antioxidant potency of PJ. Whereas antioxidant potency in vitro may not always correlate with antioxidant effects in humans, research with PJ (*1–6, 23–25*) and red wine (*28, 29*) suggests that these two potent antioxidant beverages do have effects in humans including anti-inflammatory effects.

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Title- Effects of Bing Sweet Cherries on the Concentrations of Circulating and Ex Vivo Produced Markers Associated with Cardiovascular Disease, Blood Clotting, Immune Status, Insulin Resistance, and Diabetes, in Healthy Men and Women.

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Objectives- The overall goal of our research is to determine the effect of foods rich in anti-oxidant nutrients on prevention and reversal of chronic inflammatory diseases including cardiovascular disease (CVD) insulin resistance (IR), diabetes, immune status and cancer. Results from our earlier studies showed that Bing sweet cherries had strong anti-oxidant and anti-inflammatory effects in healthy men and women.

The **specific aims** for the studies proposed here are to:

1. Determine the effects of cherries supplementation in healthy men and women on the circulating concentrations of markers associated with CVD, IR, diabetes, immune status and cancer (Please see attached MAP, Version 1.6 that lists the 89 antigens whose concentrations will be determined). We **hypothesize** that cherries will decrease the circulating concentrations of a number of markers for inflammation, blood clotting, diabetes, and cancer. Those will also improve immune status, and the concentration of anti-inflammatory markers.
2. Determine the effects of cherries supplementation on the ex vivo secretion of pro-inflammatory and anti-inflammatory markers produced by T cells and monocytes stimulated with PHA and LPS, respectively (Please see attached MAP, Version 1.1 that list the 46 antigens whose concentrations will be determined).

Analysis of plasma samples will provide information regarding the basal level of antigens and thus have physiologic relevance to normal health status. Information obtained from different samples will complement each other.

Background- We have conducted two studies with the Bing sweet cherries. The first showed that within three hours of an acute bolus, cherries reduced uric acid, oxidative stress

and inflammation (Jacob et al 2003). Results of our second study confirmed the anti-inflammatory effects of consuming cherries for 28 days (Kelley et al 2006). These results showed that cherry consumption significantly decreased circulating concentrations of a number of inflammatory markers including C-reactive protein (CRP), nitric oxide (NO) and normal T-cell expressed and secreted (RANTES). A number of other pro-inflammatory and anti-inflammatory factors also changed after cherries supplementation, but did not attain statistical significance. We also have additional plasma and tissue culture media (whole blood cultures treated with PHA or LPS) for all 5 blood draw days (d 0, 7, 21, 35 and 64) from this study stored at -70 C, which can be used for additional analyses.

Rationale- We used ELISA, Multiplex, and protein arrays to determine the serum/plasma concentrations of the inflammatory markers for the papers published. ELISA and Multiplex assays are reliable for determination of antigen concentrations ranging in the high pico- to nanograms per mL, but are not adequately sensitive for the determination of concentrations in the low picograms per mL range. Protein array that we used is sensitive to concentrations in low picograms but has very high background which is several times higher than the concentration of most antigens in plasma. Furthermore, because of its high cost we pooled the plasma samples which reduced the number of samples and the power to detect significant effects. We obtained reasonable information from using these techniques, but could have obtained much more information if we had used higher sensitivity assays on individual samples. Unfortunately, such sensitive assays were not available at the time of our analysis; however, highly sensitive quantitative protein arrays are now available, but we do not have funds to repeat these analysis. Rules Based Medicine (RBM) offers two highly sensitive assays for antigens in plasma/serum (Human MAP Version 1.6) and cell culture medium (MAP Version 1.1). MAP 1.6 analyzes for a total of 89 antigens that include pro- and anti-inflammatory cytokines, growth factors, adhesion molecules, clotting factors, hormones, markers for immune status including allergies, and cancer. MAP 1.1 analyzes a total of 46 antigens with a focus on pro- and anti-inflammatory factors, along with many others (Please see the attached for antigens on each list). Preliminary results from our other two recent studies showed that 82 of the 89 antigens in the plasma of healthy subjects could be quantified. We believe an analysis of plasma and tissue culture samples stored from our previous study with the latest technology will provide additional novel information. It would

reveal several more markers whose concentration may have been altered by cherries. This would further document the additional health benefits of cherries which would add to their marketing and cultivation. Ultimately it would help both the consumer and the cherry industry.

Proposed Analyses- We propose to analyze the plasma samples with MAP Version 1.6 for all blood draw days for all 18 subjects (90 samples total). We collected two samples prior to supplementation with cherries (day 0 and 7), two during the consumption of cherries (day 21 and 35) and one sample 28 day after the discontinuation of cherries (day 64). All these samples are important, but if funds were limited, significant publishable information could be obtained by just analyzing samples from day 7, 35 and 64 (toal of 54 samples).

We also cultured diluted blood samples without the mitogens (control) or two different concentrations (sub-optimal and maximum) of PHA (activates T cells) for 48 h. Similarly, we cultured diluted blood cells with LPS (activates monocytes) for 24 h. After treatment, cell culture media were collected and stored in -80ºC until analysis. The original intention was to analyze all collected media samples for various antigens, but considering the high cost of analyses, we plan to test antigen concentrations only in the cell culture media collected from cells stimulated with the sub-optimal concentrations of the mitogens. We expect that media collected from cells without stimulation (control) is likely to have very low concentrations of the antigens tested, and media collected after maximal stimulation may or may not show the treatment effects . We believe the best chance to see the effects of cherries would be in the media collected from cells stimulated with the sub-optimal concentration of the mitogens. For the same reasons as explained for the plasma we need to test media samples from at least days 7, 35, and 64 (preferably day 0 and 21 also). Thus, we need to test a minimum of 54 sample from cells treated with PHA and 54 for those treated with LPS.

Budget: Cost of running MAP, Version 1.6 for plasma samples is $ 500 per sample and that of MAP, Version 1.1 for cell culture media is $ 250 per sample

54 plasma samples, Human MAP Version 1.6	= $ 27,000
54 LPS Media samples, Human MAP Version 1.1	= $13,500
54 PHA Media samples, Human MAP Version 1.1	= $ 13, 500
Total	=$ 54, 000
(If all 90 samples for each treatment were analysed	=$ 90, 000)

The budget may appear high for the analysis of these samples. However, these are priceless, unthawed samples. If we were to conduct the study again to re-collect these samples, the cost will be several folds greater. Furthermore, we are not asking any funds to pay for the salary of the PI (Dr Kelley) or my support scientist (Dr YurikoAdkins), who keeps track of all samples and data, or the statistician (Dr Bruce Mackey) who has supported my studies from the beginning and has agreed to analyze the data obtained from this RBM testing. Our agency, ARS charges 10 % overhead on all incoming funds. If you do not want to pay the overhead charges, RBM can bill the Cherry Board directly. If the funds are going to come through the ARS, I would like to add additional $ 15,000 to pay for one of my Ph D students who would help with data analysis and manuscript preparation. I am hesitant to ask for the student help, however, if I can pay part of their stipend, the department matches it. If I can not match it they get nothing. If you think this is unreasonable please feel free to remove that.

Time line

We can send the samples within 2 weeks of approval from Cherry Board; RBM usually has a turn around time of 2 weeks. Data analysis, manuscript preparation for peer-reviewed journal submission could be completed within a year.

Human MAP
Version 1.6

Antigens		Autoimmune	Infectious Disease	
1. Alpha Fetoprotein	46. IL-4	90. ASCA	133. Adenovirus	176. Parainfluenza 2
2. Alpha-1 Antitrypsin	47. IL-5	91. β-2 Glycoprotein	134. *Bordetella pertussis*	177. Parainfluenza 3
3. α-2 Macroglobulin	48. IL-6	92. C1q	135. *Chlamydia pneumoniae*	178. Polio Virus
4. Adiponectin	49. IL-7	93. Centromere Prot. B	136. *Chlamydia trachomatis*	179. RSV
5. Apolipoprotein-A-1	50. IL-8	94. Collagen Type 1	137. Cholera Toxin	180. Rubella
6. Apolipoprotein-CIII	51. IL-10	95. Collagen Type 2	138. Cholera Toxin β	181. Rubeola
7. Apolipoprotein-H	52. IL-12 p40	96. Collagen Type 4	139. *Campylobacter jejuni*	182. Streptolysin O
8. BDNF	53. IL-12 p70	97. Collagen Type 6	140. Cytomegalovirus	183. Tetanus Toxin
9. β-2 Microglobulin	54. IL-13	98. Cyto P450	141. Diphtheria Toxin	184. *T. pallidum* 15kd
10. C Reactive Protein	55. IL-15	99. ds DNA	142. Epstein-Barr NA	185. *T. pallidum* p47
11. Calcitonin	56. IL-16	100. Histone	143. Epstein-Barr EA	186. *T. cruzi*
12. Cancer Antigen 19-9	57. Insulin	101. Histone H1	144. Epstein-Barr VCA	187. Toxoplasma
13. Cancer Antigen 125	58. Leptin	102. Histone H2A	145. *Helicobacter pylori*	188. Varicella zoster
14. CEA	59. Lipoprotein (a)	103. Histone H2B	146. HBV Core	
15. CD 40	60. Lymphotactin	104. Histone H3	147. HBV Envelope	
16. CD40 Ligand	61. MCP-1	105. Histone H4	148. HBV Surface (Ad)	
17. Complement 3	62. MDC	106. HSC-70	149. HBV Surface (Ay)	
18. CK-MB	63. MIP-1α	107. HSP-32	150. HCV Core	
19. EGF	64. MIP-1β	108. HSP-65	151. HCV NS3	
20. ENA-78	65. MMP-2	109. HSP-71	152. HCV NS4	
21. Endothelin-1	66. MMP-3	110. HSP-90α	153. HCV NS5	
22. ENRAGE	67. MMP-9	111. HSP-90β	154. Hepatitis A	
23. Eotaxin	68. Myeloperoxidase	112. Insulin	155. Hepatitis D	
24. Erythropoietin	69. Myoglobin	113. JO-1	156. HEV orf2 3KD	
25. Factor VII	70. PAI-1	114. Mitochondrial	157. HEV orf2 6KD	
26. FABP	71. PAP	115. Myeloperoxidase	158. HEV orf3 3KD	
27. Ferritin	72. PAPP-A	116. Pancreatic Islet Cells	159. HIV-1 p24	
28. FGF-basic	73. SGOT	117. PCNA	160. HIV-1 gp41	
29. Fibrinogen	74. SHBG	118. PM-1	161. HIV-1 gp120	
30. G-CSF	75. PSA, Free	119. PR3	162. HPV	
31. GST	76. RANTES	120. Ribosomal P	163. HSV-1/2	
32. GM-CSF	77. Serum Amyloid P	121. RNP-A	164. HSV-1 gD	
33. Growth Hormone	78. Stem Cell Factor	122. RNP-C	165. HSV-2 gG	
34. Haptoglobin	79. TBG	123. RNP	166. HTLV-1/2	
35. ICAM-1	80. Thrombopoietin	124. Scl-70	167. Influenza A	
36. IFN-gamma	81. TIMP-1	125. Smith	168. Influenza A H3N2	
37. IgA	82. Tissue Factor	126. SSA	169. Influenza B	
38. IgE	83. TNF-α	127. SSB	170. *Leishmania donovani*	
39. IGF-1	84. TNF-β	128. T3	171. Lyme disease	
40. IgM	85. TNF RII	129. T4	172. Mumps	
41. IL-1α	86. TSH	130. Thyroglobulin	173. *M. pneumoniae*	
42. IL-1β	87. VCAM-1	131. tTG (Celiac Disease)	174. *M. tuberculosis*	
43. IL-1ra	88. VEGF	132. Thyroid microsomal	175. Parainfluenza 1	
44. IL-2	89. vWF			
45. IL-3				

Plasma or serum volume requirements: 100 µl
Other fluids: 150 µl
Turnaround time: 2 weeks

RBM

TruCulture™ MAP, version 1.1

1. Alpha-1 Antitrypsin
2. Alpha-2 Macroglobulin
3. Beta-2 Microglobulin
4. Brain-Derived Neurotrophic Factor
5. C Reactive Protein
6. Complement 3
7. Eotaxin
8. Factor VII
9. Ferritin
10. Fibrinogen
11. GM-CSF
12. Haptoglobin
13. Intercellular Adhesion Molecule-1
14. Interferon gamma
15. Interleukin 1 alpha
16. Interleukin-1beta
17. Interleukin-1 receptor alpha
18. Interleukin-2
19. Interleukin-3
20. Interleukin-4
21. Interleukin-5
22. Interleukin-6
23. Interleukin-7
24. Interleukin-8
25. Interleukin-10
26. Interleukin-12 p40
27. Interleukin-12 p70
28. Interleukin-15
29. Interleukin-17
30. Interleukin-23
31. Matrix metalloproteinase type 2
32 Matrix metalloproteinase type 3
33. Matrix metalloproteinase type 9
34. Macrophage Inhibitory Protein 1 alpha
35. Macrophage Inhibitory Protein-1 beta
36. Monocyte Chemotactic Protein-1
37. RANTES
38. Stem Cell Factor
39. Tissue Inhibitor of Metalloproteinase
40. Tumor Necrosis Factor alpha
41. Tumor Necosis Factor beta
42. Tumor Necrosis Factor receptor alpha 2
43. Vascular Cellular Adhesion Molecule type 1
44. Vascular Endothelial Growth Factor
45. von Willebrand's Factor
46. Vitamin D Binding Protein

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Title: Role of Fresh Cherries in Modulating Biomarkers of cancer Risk among Males at risk for Prostate Cancer.

Background:Prostate cancer (PCa) is diagnosed in ~185,000 men with ~29,000 PCa related deaths in the U.S. annually and is the most common cancer in males (1). In most men, PCa is a manageable indolent disease whose treatment carries a high morbidity rate (incontinence and impotence). PCa remains a disease whose diagnosis and treatment confer questionable benefit for some men, particularly elderly men. Not surprisingly, an estimated 30% of men use some complementary approach to their prostate care including vitamin supplementation and diet intervention [1]. Low grade chronic inflammation has been implicated as a risk factor in prostate related pathologies including benign hyperplasia and cancer [2]leading to high interest in pharmaceutical and nutrients with anti-inflammatory properties[1]. The identification of dietary micronutrients that reduce risk through targeted effects on inflammation offers an attractive model for prostate disease and cancer prevention. Anthocyanin, a potent anti-oxidant found at high levels in cherries and berries has been shown to inhibit cylooxygenase-2 (COX-2) and PGE 2 production in keratinocytes [3] and to alter the TNF-alpha response in endothelial cells [4]as evidence of potent anti-inflammatory activity through action on the NFKappa B potent proinflammatory pathway. Here we propose a Phase I study of the effects of high anthocyanin exposure through daily consumption of 3 cups of cherries on biomarkers of inflammation as intermediate surrogate markers related to prostate and men's health.

Hypothesis: The average man at risk for PCa who consumes 3 cups of fresh cherries daily (high anthocyanin) for 4 weeks will show a significant reduction in select biomarkers of inflammation (urinary PGE2M, 8-epi-PGE2,11-dtxB2, hsCRP) compared to men consuming a diet low in anthocyanin intake.

Methods and Experimental Design:

<u>Study population</u>: Healthy men who are screened negative for active PCA but who meet any of the following criteria and who have baseline hsCRP levels above the population mean will be considered eligible for participation in this study. Elevated risk for PCA for this study is defined as any of the following:

1. Age $\geq$ 70 years or
2. A family history of PCA or
3. A persistent elevation of PSA (>4ng/ml) with negative biopsy or
4. Presence of high-grade prostatic intraepithelial neoplasia or
5. Prostatitis or
6. Benign hyperplasia

It is expected that the majority of study subjects meeting these criteria will be over age 65 years; further. 30 subjects will be recruited for this Phase I. A sample size of 30 is chosen to establish estimates of variances, correlations, and/or differences in the biomarkers for use in power

calculations that will guide selection of a sample size for a larger full-scale study of cherries and inflammatory processes of relevance to prostate health in men.

Study design: Limited data exist regarding the appropriate dose of sweet cherries to use in human feeding studies for the promotion of biologically favorable anti-inflammatory responses. One trial suggested 2.5 cups/day for 4 weeks, but this has not been replicated [5]. Here we propose to complete a 4-week feeding study in 30 men whose baseline hsCRP is above the population mean and who meet any of the above criteria including age of 70 as the only qualifying criteria. Specifically, after a 2-week no anthocyanin diet and non-steroidal anti-inflammatory wash-out period with the exception of low dose daily aspirin for heart health, subjects will be provided 3 cups of cherries daily for 4 weeks. Urinary and serum samples will be collected pre-washout(screen hsCRP for eligibility), post-washout and at 4 weeks with consumption of last dose in the clinic 1 hour prior to blood draw and urine collection for anthrocyanin measures. The outcome markers selected for investigation include serum hsCRP (a measure of chronic low grade inflammation), urinary PGEM (13,14-dihydro-15-ketometabolites, a stable metabolite measure of cyclo-oxygenase 2 activity)[6], urinary 8-epiPGF2α[7] (F_2-isoprostane 8-iso prostaglandin $F_{2\alpha}$; a non COX2 measure of bioactive products of lipid peroxidation) and urinary 11-dtxB2 (a major enzymatic metabolite of thromboxane A_2 and marker of platelet aggregation) have been previously shown to be modulated by non steroidal anti-inflammatory drugs and food components in human trials. In addition, we plan to measure anthocyanin levels in a 24 hour urine sample at all three collections (pre washout, post washout (baseline) and end of study (4 week). We have included measurement of plasma homocysteine to monitor potential adverse effects of high daily consumption of cherries. Nakagawa et al.,[8] found that rats exposed to anthocyanin through the diet suffered elevated homocysteine levels that were attributed to anthocyanin effects on the metabolic regulation of sulfur amino acids and S-adenosyl methionine. Such elevation in humans would be considered a potential dose limiting toxicity as elevated homocysteine is associated with increased disease risk in humans[9].

For each biomarker a change from baseline (post-washout) to end intervention of > 1 standard deviation will be considered a biologically important response and will thus inform the need to advance to a larger, dose-response study. A null hypothesis will be defined as a response rate of <5% while a significant response rate will be defined as > 20%. An effect size >20% is selected as it is more likely to reflect a true change and not simply attributable to the combined effects of assay and individual variances throughout the conduct of the study.

Expected Outcome

Future Phase II dose-specific intervention trial - Based on the effect size and response rate (number of subjects with significant change in biomarkers of the total subjects studied) derived from the Phase I study, funds will be sought to conduct a larger Phase II dose-response study in which men with prostate symptoms will be randomized to low anthocyanin background diet, with the addition of 3 cups cherries/day or 1.5 cups cherries/day. The number of men to be enrolled and the duration of intervention will be determined by the results of the Phase I portion of the trial.

Significance Men diagnosed with PCA and those with prostate health related issues including prostatitis and benign prostate hyperplasia may benefit from the consumption of diets enriched

in food items, such as sweet cherries, that increase exposure levels to anthocyanins and potentially other synergistically acting bioactive cherry components. Demonstrating a health benefit of sweet cherries in the form of effects on proinflammatory factors is likely to result in an increase in consumption of sweet cherries in high risk men. Sweet cherries represent a commonly preferred but somewhat expensive fruit source in the diet. Increasing the health value may diminish concerns about the higher overall costs of this potentially health-promoting fruit selection. Further, if shown to be advantageous to prostate health, sweet cherry producers could consider the development of alternative sweet cherry products to promote regular consumption.

Potential limitations Because our primary hypothesis relates to inflammation and effects on inflammatory markers, the preferred study population is one that does not regularly use anti-inflammatory medications (e.g. aspirin, celecoxib, etc). The target study population is likely to include some men who are considered to be regular users of NSAIDS. We will allow regular use of low dose aspirin as the subject's normal behavior but ask the men to refrain from NSAID use during the washout and during the trial and report any use of NSAIDs while on study. A two week washout period should be sufficient to return the study biomarkers to a stable baseline value prior to cherry feeding.

Budget:

Personnel

P. Thompson, Ph.D. Research Assistant Professor, Department of Pathology and member of the Arizona Cancer Center will oversee all aspects of the study. Dr. Thompson has expertise in the conduct of phase I cancer prevention studies and is an expert in biomarker measures in urine and serum. 5% salary + FTE is request for 12 months at $6,000.

Research Assistant (TBD). Salary is requested for 0.25 FTE for a research assistant for 12 months to process all biologic samples and to conduct serum and plasma biomarker measures. 25% salary + FTE is estimated at $12,000

Trial Coordinator/Recruiter (TBD) Salary is requested for 0.5 FTE trial coordinator/recruiter to conduct daily aspects of the trial for 12 months to include recruitment, collection of all biologics, scheduling and monitoring of subjects, and collection of any adverse events. The trial coordinator will work with Dr. Thompson to complete the IRB protocol, initiate the study, liaison with local urologist to identify eligible subjects and generally coordinate the study. It is envision that a member of our current Prostate Cancer Prevention Research Team will be recruited and bring existing expertise and community contacts to conduct the planned study. 50% salary + FTE is estimated at $30,000.

Supplies A number of measures of serum, plasma and urine markers are planned. The costs are estimated below by test. The hsCRP screen will be conducted on all potentially eligible men. Given the criteria we have selected and the high level of obesity in the target population, we estimated that we will have to hsCRP screen 3 men for every 2 that will be eligible. This means that the initial number of hsCRP tests will be 45 for the 30 men recruited to the study.

	Pre study	Post washout	End of study	Cost of test
Serum hsCRP	45	30	30	25$ x105=$2625
Plasma Homocysteine		30	30	45$x60=$2700
Urinary PGEM		30	30	10$x60=$600
Urinary 8-epi-PGE2		30	30	10$x60=$600
Urinary 11-dtxB2		30	30	10$x60=$600
Urinary anthocyanin		30	30 x 1	25$x60=$1500
			TOTAL	$8625

Other costs: monies are requested to cover the cost of phlebotomy supplies and other general lab consumables necessary to complete the biomarker studies. These are estimated at $750

Total costs are estimated at $69,500 for 12 months.

The study timeline includes 2-3 months to initiate the study that includes submitting and receiving IRB approval and identifying the first 15 subjects for the study to start in months 3-4. The second group of 15 men will be accrued and initiated at the end of the collection period for study group 1. With the washout phase (2 weeks) and then 4 week feeding, the collection of biologics is anticipated to be done by month 7 at the latest. That allows 2 months for batch biomarker analyses appropriate to small studies to reduce run to run variance. Data analysis will commence in months 10-12 with the intent to complete a draft manuscript immediately following completion of the analyses.

12 month Projected Timeline of Work and Activities by Months	
Months	Activities
1-3	IRB protocol and approval process, engagement of urology centers for recruitment of subjects, build data base for biospecimen collection, tracking and biomarker results
3-5	Conduct feeding in first group of 15 men 2 week washout followed by 4 week feeding collect biologics on all men at three time points, send out hsCRP and homocysteine at time of draw recruit next group of 15 men for group 2
5-7	Conduct feeding in second group of 15 men 2 week washout followed by 4 week feeding, send out hsCRP and homocysteine at time of draw collect biologics on all men at three time points
8-10	Conduct biomarker analyses in batch QC and clean all data for analysis
9-12	Conduct change analysis for all biomarkers and summarize results for publication and presentation at scientific meetings

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