CALIFORNIA CHERRY ADVISORY BOARD
WASHINGTON STATE FRUIT COMMISSION

Joint Strategic Planning Session

&

Scientific Advisory Board Meeting

Lodi, CA
March 2, 2010

California Cherry Advisory Board
Washington State Fruit Commission

California Cherry Advisory Board & Washington State Fruit Commission
Joint Industry and Scientific Advisory Board Meeting

March 2, 2010

Lodi, CA

Introduction:

The California Cherry Advisory Board (CCAB) and the Washington State Fruit Commission (WSFC) first met with March 2006 to initiate a Comprehensive Industry Strategic Plan (CISP) for the US fresh sweet cherry industry. CCAB and WSFC met again in January 2008 to review the progress made to date and to determine next steps. From this meeting, industry formed a Health & Nutrition Committee (HNC) to develop and execute a health research strategy for fresh sweet cherries. This effort included the formation of a Scientific Advisory Board (SAB). Members of the SAB include:

Darshan S. Kelley, Ph D
Research Chemist/Adjunct Professor
Western Human Nutrition Research Center, ARS, USDA
Department of Nutrition, University of California at Davis

Cheryl L. Rock, PhD, RD
Professor
Department of Family and Preventive Medicine
School of Medicine
University of California, San Diego

Cynthia Thomson PhD, RD
Consulting SAB Director
Associate Professor
Nutritional Sciences and Medicine

Andrew Breksa
Research Chemist & Lead Scientist
USDA, ARS, WRRC

The SAB held its first meeting in March 2009 to start answering 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?

During the meeting, eight recommendations for future research emerged. These were prioritized based on the need to build a foundation for understanding and growing that understanding with each successive research project. However, some of these projects can be conducted at the same time if funding is available. The following summarizes key recommendations:

1. Rules Based Medicine Study *Cost: $115,500*
2. Develop a Standardized Product to Aid Future Research *Cost: Unknown*
3. Chemical Analysis of the Fruit *Cost: $20-30K + admin mark-up*

4. Dose Response Study — *Cost: $60-80K + admin mark-up*
5. Feeding Trials — *Cost: roughly $100K and up*
6. Gene Array Sample — *Cost: $20-40K + admin mark-up*
7. Epidemiological Study – Retro Data Analysis — *Cost: $50K+ admin mark-up*
8. Clinical Studies — *Cost: significant*

The complete report from the SAB meeting inlcuding additional information about each of these recommendations is provided in this meeting packet.

The SAB and delegates from the California Cherry Adivisory Board and the Washington State Fruit Commission are reconvening on March 2, 2010 to continue defining health research needs and to address other areas of concern to the sweet cherry industry. The following outlines specific details for the meeting inlcuding location, objectives, and agenda.

Meeting Location:

Holiday Inn Express
1337 East Kettleman Lane
Lodi, CA 95240
Phone: 209-210-0150 Fax 209-369-7629

Objectives:

Key objectives of the meeting are two fold. For the SAB portion, industry is seeking to better understand:

- Expected outcomes of the Rules Based Medicine study and how industry can communicate those results
- The merits of a proposed study on diabetes and whether industry should pursue this research
- What, if any, tart cherry research can be extended to sweet cherries and what additional research on sweet cherries might be necessary to do so
- If industry's current research plan remains relevant and if so, what should be the next steps

For the joint industry meeting, CCAB and WSFC would like to: :

- Review global production of sweet cherries to better understand current and future supply and how the California and Northwest industries fit into that supply picture
- Discuss packing logistics to address larger crops
- Understand threats posed by Spotted Wing Drosophila and how industry will address these threats
- Identify other areas of concern or opportunities for cooperation

Agenda:

8:00AM – 8:30	Continental Breakfast and set-up
8:30 – Noon	**SAB Meeting**
8:30 – 8:45	Welcome, Introductions, and Meeting Expectations (BJ Thurlby and Jim Culbertson)
8:45 – 9:00	Overview of Key Findings from last SAB Meeting (Mike Rucier)
9:00 – 9:30	Rules Based Medicine Presentation and Discussion (Dr. Kelley)
9:30 – 10:15	Postprandial metabolism and type 2 diabetes mellitus study discussion (Dr. Thomson discussion leader)
10:15 – 10:30	Break
10:30 – 11:30	Tart cherry research review and relevancy to sweet cherries (Dr. Thomson discussion leader)
11:30 – 12:00	Review current research plan and revise if necessary (All SAB Members)
12:00 – 2:30	Lunch and Industry Tour Waterloo Restaurant 10447 E. Waterloo *(Hwy 99 to Waterloo Rd East 6 miles)* Stockton, CA (209) 931-4019
3:00 – 5:30	**Industry Meeting**
3:00 – 3:20	Global cherry supply overview (Mike Rucier) *Focus on Spain, Turkey, and China*
3:20 – 3:50	Future US crop scenarios (BJ Thurlby and Jim Culbertson)
3:50 – 4:15	Packing logistics: augmenting NW line capacity (BJ Thurlby and Jim Culbertson)
4:15 – 4:45	Spotted Wing Drosophila: situation status and next steps (Jim Culbertson)
4:45 – 5:15	Other discussion
4:15 – 5:30	Summary and Conclusions (Mike Rucier)
7:00	Dinner Giusti's Place 14743 Walnut Grove-Thornton Road Walnut Grove, CA 95690 Tel: 916-776-1808

REPORT ON THE FIRST MEETING OF THE SWEET CHERRY INDUSTRY'S SCIENTIFIC ADVISORY BOARD

INTRODUCTION:
The California Cherry Advisory Board (CCAB) and the Washington State Fruit Commission (WSFC) representing the Northwest cherry industry held the first meeting of its newly formed Scientific Advisory Board (SAB) on March 2, 2009 in Seattle, WA. The purpose of the SAB is to help the sweet 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?

Meeting participants included:

Andrew Breksa
Research Chemist & Lead Scientist
USDA, ARS, WRRC

Darshan S. Kelley, Ph D
Research Chemist/Adjunct Professor
Western Human Nutrition Research Center, ARS, USDA
Department of Nutrition, University of California at Davis

Cheryl L. Rock, PhD, RD
Professor
Department of Family and Preventive Medicine
School of Medicine
University of California, San Diego

Mike Rucier
SAB Facilitator
Manager, International Marketing
Bryant Christie Inc.

Cynthia Thomson PhD, RD
Consulting SAB Director
Associate Professor
Nutritional Sciences and Medicine
University of Arizona

Andrew Willis
Director of Domestic Marketing
Washington State Fruit Commission

B.J. Thurlby
Executive Director
Washington State Fruit Commission

MEETING FORMAT:
Although we did have a set agenda, it was followed only loosely. Instead, meeting participants were urged to speak freely on a variety of topics related to their own research, current cherry research in particular, and implications for future research efforts. Dr. Thomson initiated the discussion by providing a general overview of what is currently in the literature regarding cherry health research. Discussion ensued throughout her presentation. This was followed by a formal presentation by Dr. Kelley that provided results from his past

research on cherry health benefits, and a proposal for new research. After more discussion, several recommendations began to emerge. Those recommendations were discussed in greater depth, prioritized, and a rough estimate of costs was provided. Those recommendations are noted below.

KEY RECOMMENDATIONS:
During the discussion, eight recommendations for future research emerged. These were prioritized based on the need to build a foundation for understanding and growing that understanding with each successive research project. However, some of these projects can be conducted at the same time if funding is available. The following summarizes key recommendations:

1. Rules Based Medicine Study — *Cost: $115,500*
2. Develop a Standardized Product to Aid Future Research — *Cost: Unknown*
3. Chemical Analysis of the Fruit — *Cost: $20-30K + admin mark-up*
4. Dose Response Study — *Cost: $60-80K + admin mark-up*
5. Feeding Trials — *Cost: roughly $100K and up*
6. Gene Array Sample — *Cost: $20-40K + admin mark-up*
7. Epidemiological Study – Retro Data Analysis — *Cost: $50K+ admin mark-up*
8. Clinical Studies — *Cost: significant*

Additional information about each of these recommendations is provided below:

1. **Rules Based Medicine Study** *Estimated Cost: $115,500*
   In 2006, the University of California at Davis and Western Human Nutrition Research Center (WHNRC) conducted a feeding study on Bing cherries to determine:
   - Serum concentration of markers of inflammation
   - Blood lipids, lipoproteins, particle size and number
   - Hematology and clinical chemistry panels including insulin

   Dr. Kelley was one of the lead researchers on this project. Before eating cherries and at regular intervals after eating the fruit, research subjects had their blood drawn. The results showed that cherry consumption had significant effects on some circulating markers of inflammation, but not all that were expected. The changes were too small to attribute to cherries.

   Since this research was conducted, new methods for biomarker testing have been developed by Rules-Based Medicine (RBM), the world's leading multiplexed biomarker testing laboratory. If approved for funding, RBM under the guidance of Dr. Kelley's lab would analyze plasma and media samples collected from cultured white blood cells obtained from the previous cherry feeding study to determine the effects of the antioxidant nutrients in fresh sweet cherries on biomarkers tied to the prevention and reversal of chronic inflammatory diseases including cardiovascular disease, insulin resistance, diabetes, immune status, and cancer.

More specifically, RBM will analyze plasma and media samples using two MAP assays. The first assay, (Human MAP Version 1.6) will analyze the plasma samples for 89 antigens that include pro-and anti-inflammatory cytokines, growth factors, adhesion molecules, clotting factors, hormones, and markers for immune status including allergies, and cancer. The second assay (Human MAP Version 1.1) will analyze the media samples for 46 antigens with a focus on pro-and anti-inflammatory factors. The output of RBM's MAP assays will form the basis for the findings in this study that will be analyzed by the researchers at Dr. Kelley's lab.

It is anticipated that within two years of the initiation of the study, Dr. Kelley would likely have enough evidence to publish a paper in a noted scientific journal. The results of this research would therefore establish a better understanding of the bioactivity of sweet cherries, help to establish a direction for future feeding trials or clinical research, while giving industry a study that it can promote immediately in its public relations efforts.

While the cost is not cheap, the SAB suggested that conducting the RMB study would be the easiest way to broaden our understanding of the product and would be an important first step in industry's research efforts. The plasma samples collected from their earlier feeding trials are invaluable and it is wise to gain as much information out of those samples as possible. **The SAB therefore recommended that industry pursue this project as their top priority.**

2. **Develop a Standardized Product to Aid Future Research** *Cost: Unknown*
SAB members agreed that one of the great challenges in doing research on fruit and vegetables in general is obtaining a shelf-stable product that can be standardized and available throughout the year. While doing whole fruit research is valuable, seasonal limitations and variations in fruit bioactivity from year to year, lot to lot, and variety to variety can challenge research results and restrict when and how studies can be conducted. Therefore, **the SAB recommends the cherry industry develop a freeze-dried product** that can be used for subsequent feeding trials.

   It was mentioned that the table grape industry uses such a product in their research program. **The cherry industry should contact the California Table Grape Commission** to see if they would be willing to provide information about how they obtain their freeze-dried product, what steps they must undertake to ensure product consistency, and how they determine the relationship between shelf-life and bioactivity.

   Due to the amount of variables that must be considered and the fact that fresh samples for freeze drying can only be collected during a short window during harvest, **industry should make it a priority to develop an action plan for this project before the 2009 harvest begins**.

3. **Chemical Analysis of the Fruit** *Cost: $20-30K + admin mark-up*
Part of the process of developing a standardized, freeze-dried product as noted above includes conducting a chemical analysis of the fresh fruit. After collecting a wide variety of samples from different locations, varieties, harvest timing, etc., the

samples would undergo a chemical analysis using ORAC and FRAP to identify what compounds exist in the fruit and at what levels. Results would allow us to easily compare the chemical make-up of cherries with that of other fruits. For instance, POM says their product contains XYZ and therefore it is healthy. According to our chemical analysis, cherries have that too, so therefore cherries must also be healthy. According to the SAB, a chemical analysis of the fruit provides a basic understanding of the phytonutrients in cherries and how those nutrients might vary across a wide variety of samples. **If possible, this project should be conducted in concert with the efforts to develop a standardized, freeze dried product.**

4. **Dose Response Study** *Cost: $60-80K + admin mark-up*

Obtaining results from the chemical analysis is the first step in establishing a standardized dosage which is important for future research efforts. In particular, a dose response study gives the research community information about how much cherries a person needs to eat to achieve a certain reaction in the body. For instance, if only 30 cherries are needed to realize the result, then future feeding trials don't need to recommend 45 cherries. **A dose response study should take place only after industry completes the first three recommendations noted above.**

5. **Feeding Trials** *Cost: roughly $100K and up*

Assuming industry has done preliminary work to develop a standardized product and has established a clear dose response, feeding trials are the next step to learn more about how the body reacts to the different phytonutrients in sweet cherries. Although industry has previously conducted feeding trials without the benefit of having a more basic understanding of bioactivity and dose response, such information would likely strengthen future results. The SAB suggests that the media "loves" feeding studies, particularly if they are about a relevant topic. The SAB suggests that based on historical cherry studies, **industry should focus on arthritis, insulin resistance, and inflammation as primary areas of focus**. These are also relevant to consumers and could drive future demand if evidence linked cherry consumption to reduction or prevention of these diseases.

The SAB suggested that a researcher named Seerman NP might be a potential addition for the SAB. He has done a good deal of work on the anti-inflammatory effects of select anthocyanins in cell cultures and could help guide future feeding trials.

6. **Gene Array Sample** *Cost: $20-40K + admin mark-up*

A gene array sample looks at how gene-specific nucleic acids react to various phytonutrients. In one test, 30,000 or more biomarkers can be analyzed to determine bioactivity. There was some debate among SAB members about when and if industry should pursue such a project. There was some consensus that **for any feeding trial, industry should plan to add on gene array analysis to get the broadest understanding of how the body reacted to cherry phytonutrients**.

7. **Epidemiological Study – Retro Data Analysis** *Cost: +$50K+ admin mark-up*

According to Wikipedia, epidemiology is defined as

> …the study of factors affecting the health and illness of populations, and serves as the foundation and logic of interventions made in the interest of public health and preventive medicine. It is considered a cornerstone methodology of public health research, and is highly regarded in evidence-based medicine for identifying risk factors for disease and determining optimal treatment approaches to clinical practice.[1]

Instead of looking at the fruit and seeing what benefits it can bring to those that eat it, epidemiological studies look at a diseased population and understand how consumption of the fruit can reverse the progression. In other words, what does eating cherries do to those that suffer from arthritis?

The SAB suggests industry can conduct a retroactive data analysis on existing food diary databases. With the right database and the right team to mine the data, industry could better understand how the bodies in a certain diseased population reacted to eating cherries. Apparently the data is there, we just need to get someone that can dig it up and knows how to interpret the results.

8. **Clinical Studies** *Cost: significant*

Clinical studies are the gold standard of health research, but can be cost prohibitive. The length of the study and the amount of participants greatly impacts costs which could start at $500K and easily pass $1 million or more. Industry should consider the long-term impact that such an investment could have on future sales. However, industry should first conduct feeding trials and other pilot efforts to determine which areas could have the greatest chance of success.

If industry is willing make such a large investment, it will want to almost guarantee results. If enough preliminary evidence is gathered, the National Institutes of Health (NIH) might decide to further explore the benefits of cherries. In which case, the federal government would foot the bill for the clinical work. **Industry should therefore revisit the idea of conducting a clinical study only after it has accomplished the preliminary steps previously recommended**.

REQUEST FOR PROPOSALS AND MULTI-YEAR FUNDING:

The last bit of advice that the SAB provided during the meeting was steps industry should take to initiate specific research studies. Industry has two choices for enlisting work from the scientific community. First, if industry has a specific researcher in mind, they can engage that researcher who can then forward a study design, budget, and timeline. Second, industry can seek proposals from candidates. If industry decides to develop a Request for Proposal (RFP), it should spell out how much administrative overhead industry is willing to cover, and should be as specific as possible in the project objective. Ideally, candidates would clearly identify the study design and methodology in their proposal which can be compared against the pool of applicants. Industry could then use the SAB to identify potential weaknesses and help improve the design of those researchers that might be selected.

[1] <http://en.wikipedia.org/wiki/Epidemiology> Accessed on March 10, 2009

Perhaps the most critical bit of advice from the SAB was to ensure that researchers are given the opportunity to conduct multi-year projects. Most research cannot be completed in one year and have a research budget that spans more than one year would give researchers greater flexibility in the types of research they pursue and even the quality of the research. **Therefore, it is recommended that industry establish a multi-year budget for health research that is insulated from annual budget swings that might occur due to varying crop situations**. Industry must have a long-term view of health research and must be willing to fund it accordingly.

ADDITIONAL SAB MEMBERS AND FUTURE MEETINGS:
Overall, the SAB seemed positive about their experience and would be willing to continue serving in such a capacity. They did recommend that industry could rotate in additional SAB members depending on what level of expertise was needed at any given time. The existing SAB could prove a valuable resource in helping industry to identify additional scientific experts and guiding industry in its health research efforts.

Going forward, industry would likely engage the SAB via conference call after priorities with each respective board is established and funding is secured. Based on available resources, the SAB can help industry to further prioritize its research effort and can help guide industry in moving forward with selecting a researcher or for creating an RFP and evaluating proposals. It is anticipated that another in-person meeting later in the year would help keep all the members on the same page.

Health research and communication are important to help ensure long-term demand for sweet cherries. One of the SAB members said that industry should "back-pocket" the science, suggesting that if industry does the work now, the science will be there when we need it.

VitaCherry Highlights

	Freeze-Dried Sweet	Freeze-Dried Tart	HiActives
Anthocyanins	0.7%	0.5%	0.8%
Flavonoids	N/A	1%	N/A
Kosher	Yes	Yes	Yes
GMO-Free	Yes	Yes	Yes
Color	Red	Red	Red

VitaCherry®

USDA ORGANIC

What is VitaCherry®?

VitaCherry is a line of highly concentrated, standardized cherry based ingredients that includes:

VitaCherry Freeze-Dried Sweet: Standardized sweet cherry powder
VitaCherry Freeze-Dried Tart: Tart cherry powder standardized to flavonoids and antioxidants
VitaCherry HiActives: Cherry powder standardized to anthocyanins

Organic, drum-dried and spray-dried VitaCherry products are also available.

What are the health benefits of VitaCherry®?

The deep red color of cherries results from flavonoid antioxidants called anthocyanins. Studies of cherries suggest that anthocyanins promote healthy response to inflammatory conditions like arthritis and gout. Cherries may also protect the body against the damaging effects of free radicals, certain chronic diseases and cellular proliferation. Study results also indicate that cherries may help slow the build-up of arterial plaque by inhibiting the enzyme, cyclooxygenase (COX).

Science also suggests that anthocyanins could help reduce the risk of developing Type-2 diabetes. In one study, animal cells treated with anthocyanins from tart cherries increased insulin production up to 50% compared to unexposed cells.

What structure function claims can I make for VitaCherry®?

Structure function claims for VitaCherry could include:

Supports Healthy Immune Response
Promotes Cardiovascular Health
Promotes Healthy Brain Function
Supports Joint Flexibility and Health

Why choose antioxidants from FutureCeuticals?

FutureCeuticals offers unique products that provide a variety of antioxidants from different natural, plant-based sources. We also go beyond typical antioxidant measurements by standardizing our products to specific levels of anthocyanins and flavonoids. By offering a branded and standardized ingredient, we can help create differentiation when a product goes to market.

How can you use VitaCherry®?

VitaCherry is ideal for nutraceutical and functional food products like capsules, tablets, and ready-to-mix beverages, among others. VitaCherry provides opportunities in markets such as children's nutrition, healthy aging and inflammation management.

For More Information:
Call Toll Free: 888.452.6853 • Visit: www.futureceuticals.com • Email: sales@futureceuticals.com

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Freeze-dried Sweet Cherries

Add the finishing touch with a cherry on top!

MOMENCE, ILLINOIS - (January 21, 2010) – Make your food products sweet and complete by adding delicious Freeze-dried Sweet Cherries from Van Drunen Farms (VDF).

Cherries have been a favorite of many for centuries, from early settlers to U.S. Presidents. There are over 1,000 varieties of cherry trees in the U.S. and of those, only about 10 varieties are produced commercially. Sweet cherries are available in varieties such as Bing, Lambert and Rainier cherries. These three account for more than 95 percent of the Northwestern sweet cherry production.

Sweet cherries are a versatile fruit that goes with just about anything! These great-to-eat treats are quite healthy for us as well—they are fat-, sodium- and cholesterol-free. They are also a good source of vitamin C, potassium and antioxidants.

Cherries in general, however, have a brief harvest season and they are highly perishable. There are no worries, though, about their availability or shelf life when using the freeze-dried form of sweet cherries from Van Drunen Farms—this cherry product is sourced in the U.S. and E.U. which allows for year-round availability.

Freeze-drying removes the moisture from the fruit while preserving the natural flavor, color and nutritional value. In this convenient form, they are easy to use, economical and reconstitute immediately.

Applications for VDF Freeze-dried Sweet Cherries include cereals, snacks, granola bars, breads and muffins, and much more.

Van Drunen Farms offers Freeze-dried Sweet Cherry Products in a wide variety of sizes including whole, sliced, diced and powder form. Customization is available.

About Van Drunen Farms

Van Drunen Farms is a family-owned company, located in the fertile farmland of the Midwest. VDF is a primary processor of culinary, all-natural, and functional food ingredients, specializing in certified organic and conventional fruits, vegetables, herbs, and specialty products for the food industry. Customization is our specialty. For more information about Freeze-Dried Organic Pineapple products, contact Irv Dorn at tel: 815-472-3100 or fax: 815-472-3850, or sales@vandrunen.com

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Our E-Newsletter Email GO

Effects of Bing Sweet Cherries on Human Markers of Inflammation

Darshan S. Kelley, PhD

Yuriko Adkins, PhD

Western Human Nutrition Research Center, ARS, USDA and Department of Nutrition, University of California, Davis

Background 1

- Inflammation is a local healing response to microbial invasion or injury; blood cells & mediators
- Chronic inflammation leads to a number of human diseases including diabetes, arthritis, CVD. Blood cholesterol normal in 50% of those getting fatal heart attack, have increased inflammation
- Markers for inflammation include, CRP, SAA, inflammatory cytokines & eicosanoids & others

Background 2

- Cherry feeding claimed to reduce arthritis in humans:
  - Ludwig W. Blau (1950) – consumption of ~227 g fresh or canned cherries per day alleviated gouty arthritis
  - Jacob, R.A. et al. (2003) – consumption of cherries reduced the serum concentration of uric acid and markers of inflammation (CRP and NO) in healthy young women

Blau LW., Cherry diet control for gout and arthritis. Texas Rep Biol Med. 1950; 8:309-311.
Jacob R. et al. Consumption of cherries lowers plasma urate in healthy women. J Nutr. 2003; 133:1826-1829.

Acute Effects of Cherries on Plasma Biomarkers

Jacob R. et al. Consumption of cherries lowers plasma urate in healthy women.
*J Nutr. **2003**; 133:1826-1829*

Biomarker	Baseline	5 h
Urate (μmol/L)	214 ± 13	183 ± 15*
CRP (mg/L)	4.29 ± 2.18	3.59 ± 1.59
Nitric Oxide (μmol/L)	37.4 ± 5.2	31.6 ± 2.1

*Different from baseline, $P<0.05$

2006 WHNRC Cherry Study

Specific Aims

Determine the effects of cherry consumption on:

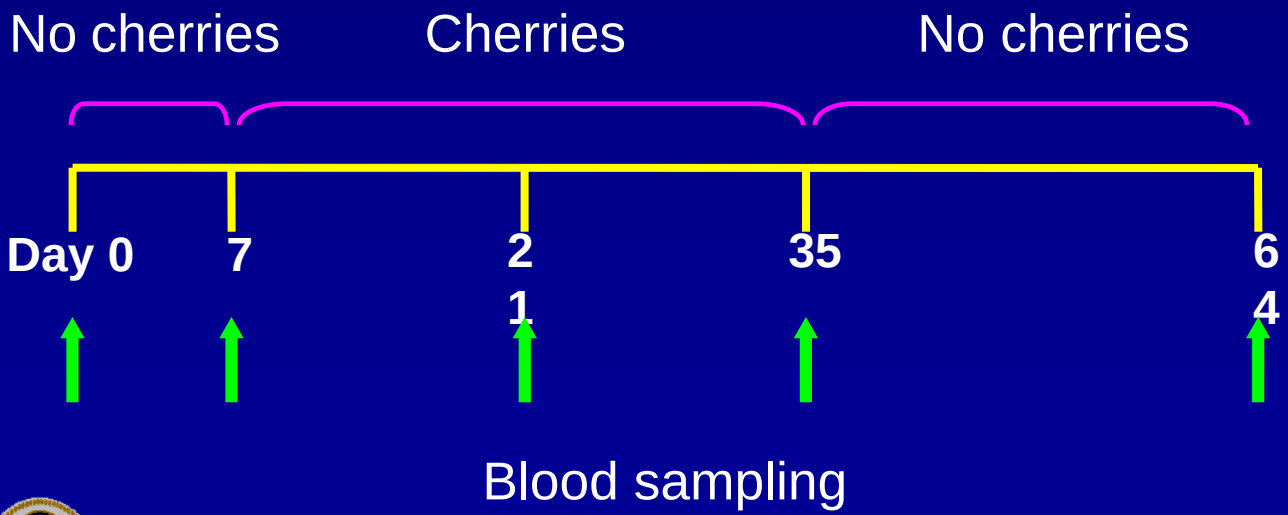
1. Serum concentration of markers of inflammation
2. Blood lipids, lipoproteins, particle size & number;
3. Hematology & clinical chemistry panels including insulin

Subject Characteristics and Study Design

2 men, 18 women

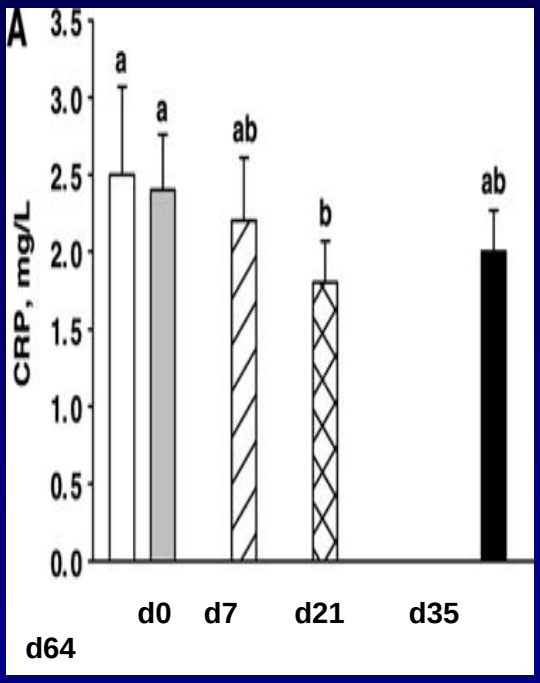
Variable	Min	Max	Mean	SEM
Age (yrs)	45	61	50	0.9
Weight (kg)	53.6	113.0	73.3	3.6
Height (cm)	150.5	186.0	166.3	2.2
BMI	19.6	30.4	26.3	0.9

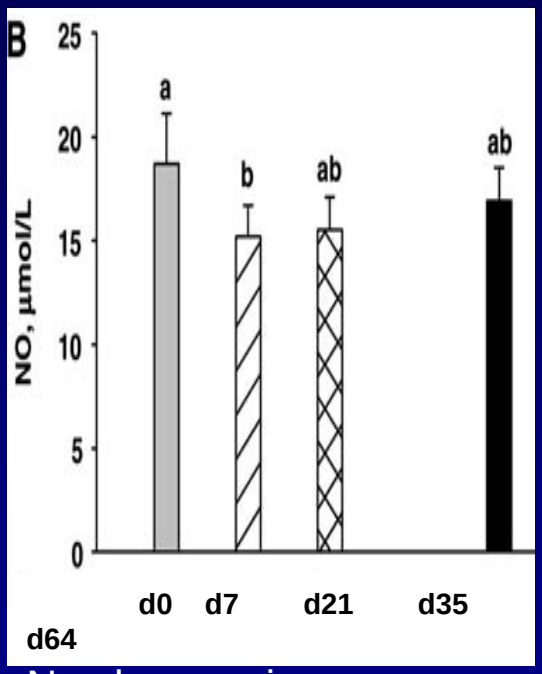
Intervention: 280 g cherries (~45 cherries)

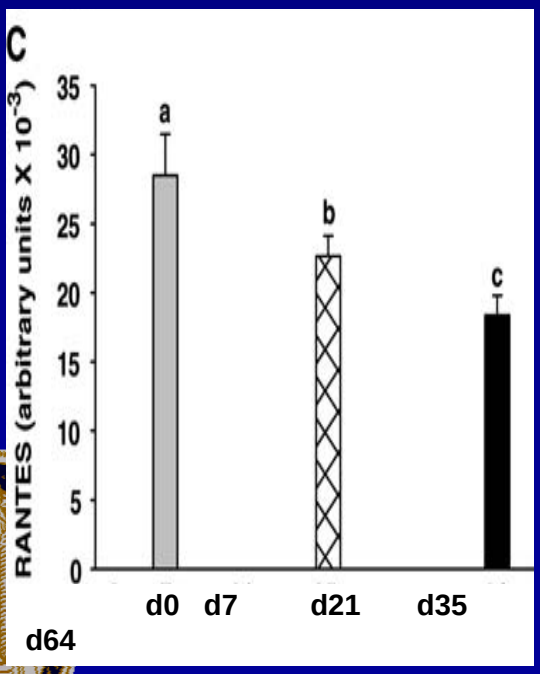


Effect Of Cherry Consumption On Circulating Markers Of Inflammation

Kelley et al. J Nut. 2006, 136:981-986







No changes in:

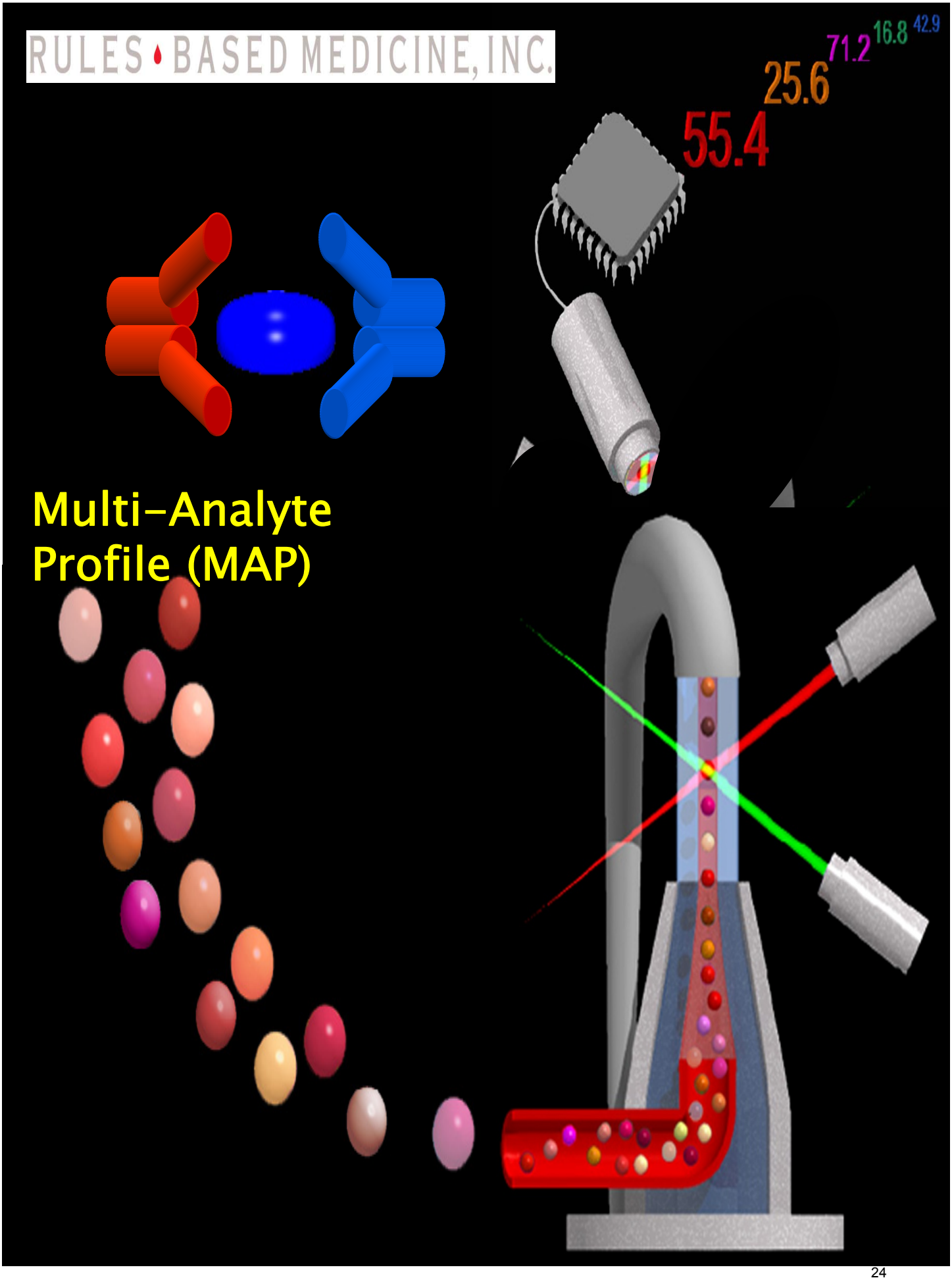
- IL-6
- ICAM-1
- TIMP-2
- Glucose
- Insulin
- Blood lipids
- Lipoprotein size and numbers
- Haematological and chemistry panels

Baylor 2008 and Other Studies

- Dr John Cush, 5/6 patients experienced noticeable relief from arthritis pain while taking capsules containing extract of cherries.
- Connolly DAJ et al 2006, tart cherry juice decreased some of the symptoms of exercise induced muscle damage
- 2 other 2009 studies; one with marathon runners and other with healthy older men and women. Both used tart cherry products (confidential information).

Why Other Markers of Inflammation Did Not Change in WHNRC 2006 Study?

- Healthy subjects with very low blood concentrations of the markers tested.
- Protein arrays used to test inflammation markers had high background, greater than the concentrations of most of the markers, except RANTES, TIMP-2, ICAM-1, IL-6sR.
- Small n of 3, instead of 20; samples for protein array pooled because of high cost.

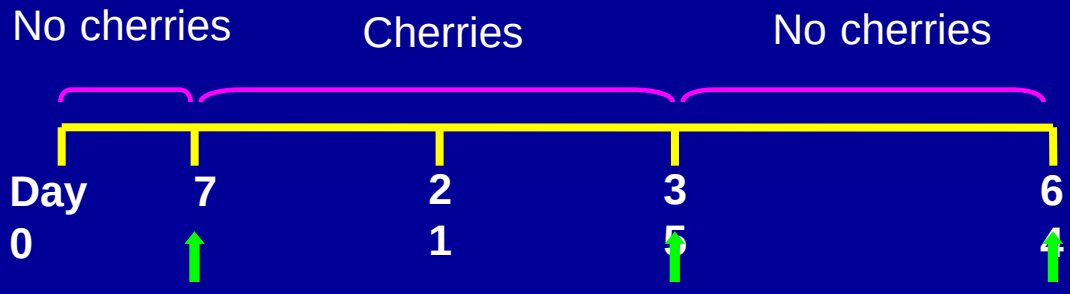

RULES • BASED MEDICINE, INC.
55.4
25.6
71.2
16.8
42.9
Multi-Analyte
Profile (MAP)

RBM
RULES • BASED MEDICINE

HumanMAP version 1.6

The Human MAP measures 89 antigens using less than 100 µL of plasma

1. Adiponectin	30. GST	60. Lymphotactin
2. Alpha-1 Antitrypsin	31. G-CSF	61. MDC
3. Alpha-Fetoprotein	32. GM-CSF	62. MIP-1 alpha
4. Alpha-2 Macroglobulin	33. Growth Hormone	63. MIP-1 beta
5. Apolipoprotein A-1	34. Haptoglobin	64. MMP-2
6. Apolipoprotein C-III	35. Immunoglobulin A	65. MMP-3
7. Apolipoprotein H	36. Immunoglobulin E	66. MMP-9
8. Beta-2 Microglobulin	37. Immunoglobulin M	67. MCP-1
9. BDNF	38. Insulin	68. Myeloperoxidase
10. C-Reactive Protein	39. IGF-1	69. Myoglobin
11. Calcitonin	40. ICAM-1	70. PAI-1
12. Cancer Antigen 19-9	41. Interferon-gamma	71. PAPP-A
13. Cancer Antigen 125	42. Interleukin-1 alpha	72. PSA, Free
14. Carcinoembryonic Antigen	43. Interleukin-1 beta	73. Prostatic Acid Phosphatase
15. CD40	44. Interleukin-1 ra	74. RANTES
16. CD40 Ligand	45. Interleukin-2	75. Serum Amyloid P
17. Complement 3	46. Interleukin-3	76. SGOT
18. CK-MB	47. Interleukin-4	77. Sex Hormone Binding Globulin
19. Endothelin-1	48. Interleukin-5	78. Stem Cell Factor
20. Eotaxin	49. Interleukin-6	79. Thrombopoietin
21. Epidermal Growth Factor	50. Interleukin-7	80. Thyroxine Binding Globulin
22. ENA-78	51. Interleukin-8	81. Thyroid Stimulating Hormone
23. Erythropoietin	52. Interleukin-10	82. Tissue Factor
24. ENRAGE	53. Interleukin-12 p40	83. TIMP-1
25. Factor VII	54. Interleukin-12 p70	84. Tumor Necrosis Factor-alpha
26. Fatty Acid Binding Protein	55. Interleukin-13	85. Tumor Necrosis Factor-beta
27. Ferritin	56. Interleukin-15	86. Tumor Necrosis Factor RII
28. Fibrinogen	57. Interleukin-16	87. VCAM-1
29. FGF-basic	58. Leptin	88. VEGF
	59. Lipoprotein (a)	89. von Willebrand Factor

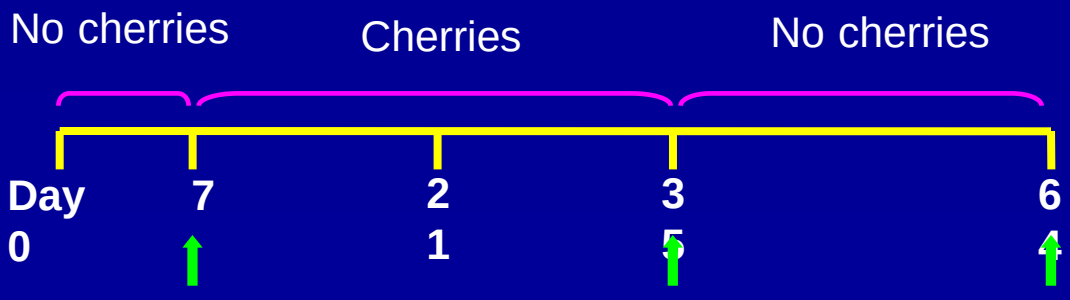


RBM
RULES • BASED MEDICINE

ruCulture™ MA version 1.1

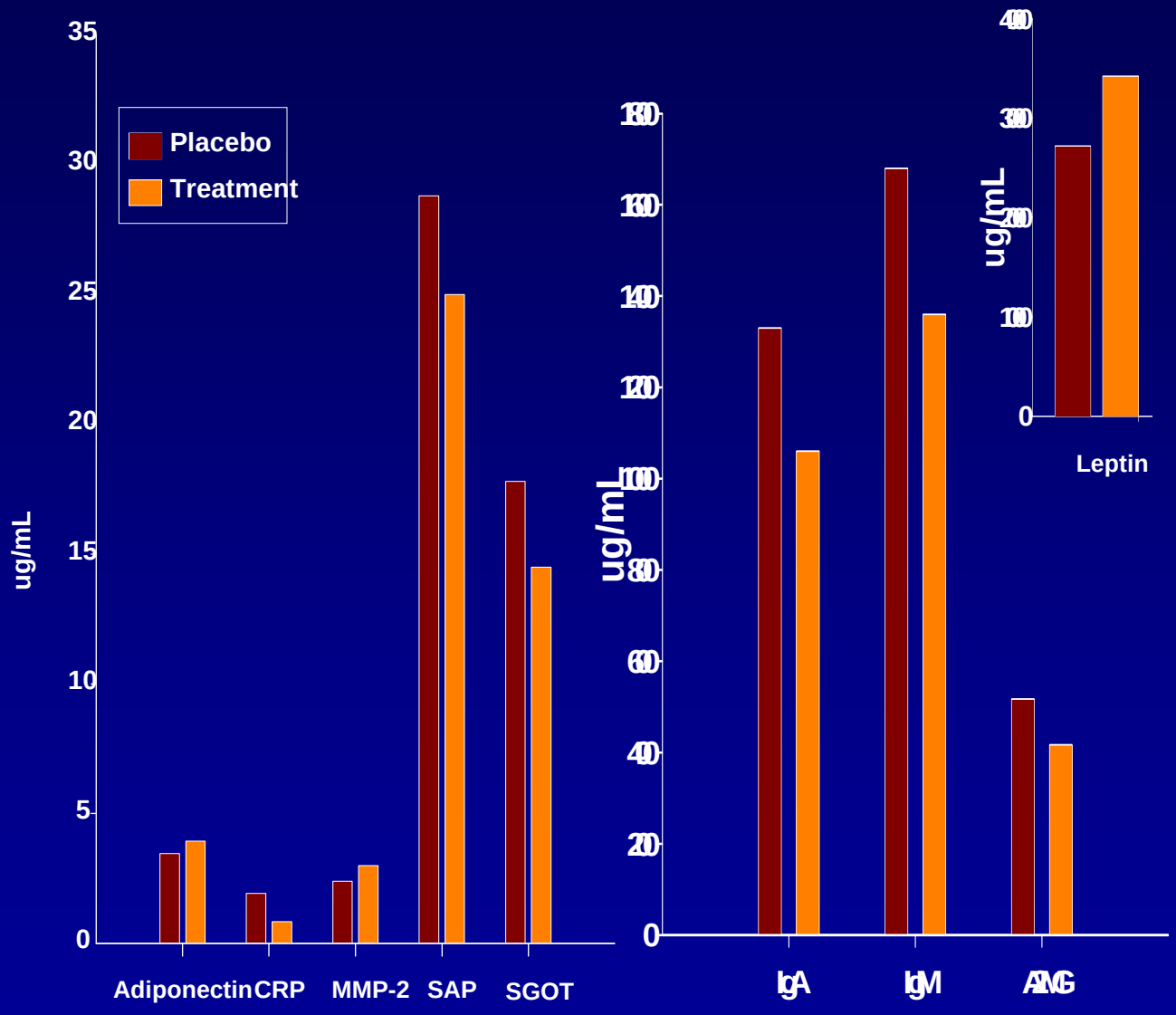
The TruCulture™ MAP measures 46 antigens using less than 250 µL of culture media

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



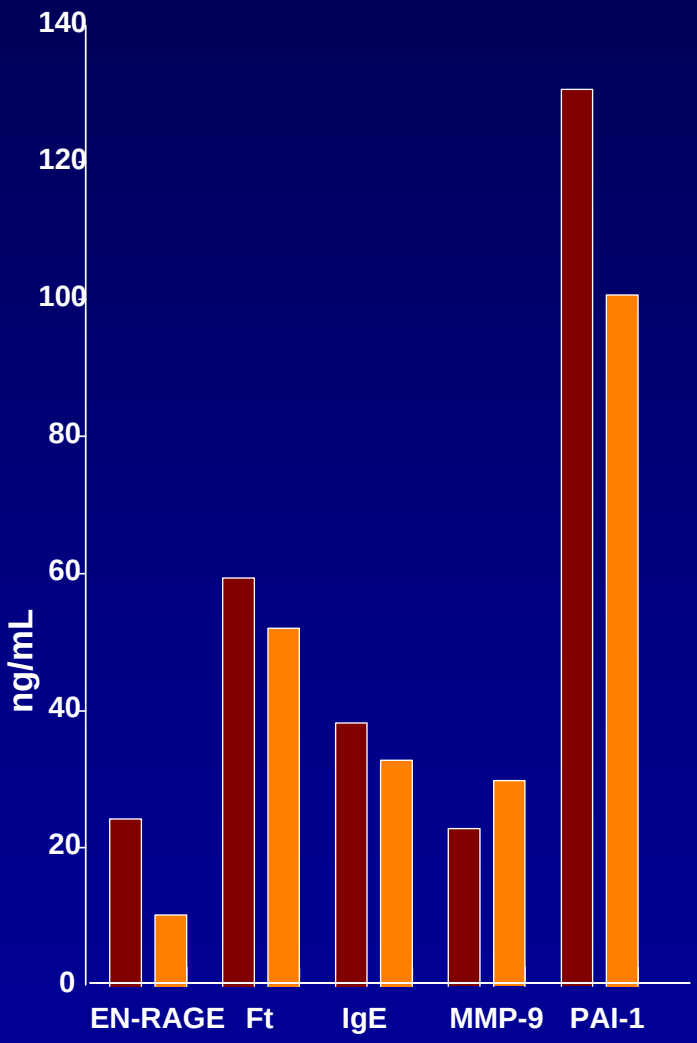
RBM Human MAP Sensitivity

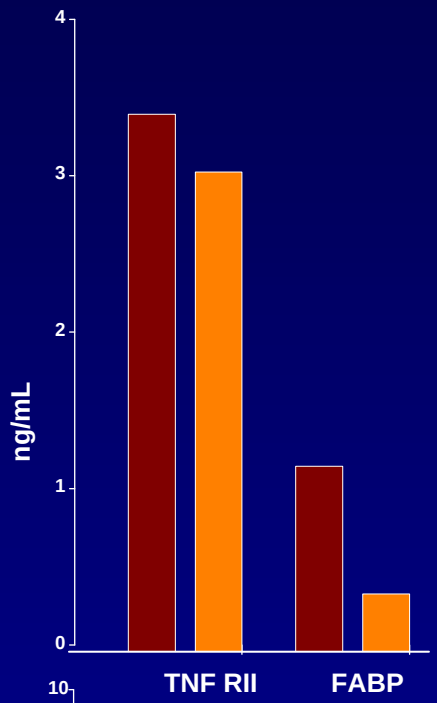
Effect of DHA on Markers of Inflammation and Insulin Resistance in Hypertriglyceridemic Men

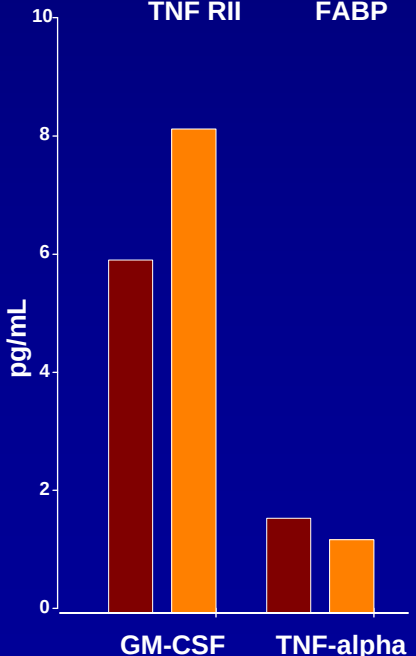


RBM Human MAP Sensitivity

Effect of DHA on Markers of, Inflammation, Infection, and lipid transport in Hypertriglyceridemic Men







Samples From 2006 Cherry Study

- Plasma samples from 18 subjects for study d 0, 7, 21, 35, and 64 (total 90 samples)
- Tissue culture media from whole blood cultures stimulated with 3 concentrations of LPS for 18 subjects for d 0, 7, 21, 35 (total 216 samples)
- Tissue culture media from whole blood cultures stimulated with 3 concentrations of PHA for 18 subjects for d 0, 7, 21, 35 (total 216 samples)
- RBM analysis for some of the above samples

Budget

3 time points (days 0, 35, 64), 18 subjects:

54 plasma samples, Human MAP Version 1.6 $27,000

2 time points (days 0, 35), 18 subjects:

36 LPS media samples, TruCulture MAP Version 1.1 $9,000

36 PHA media samples, TruCulture MAP Version 1.1 $9,000

Total $45,000

Alternative:

3 time points (days 0, 35, 64), 6 subjects:

18 plasma samples $9,000

2 time points (days 0, 35), 6 subjects:

12 LPS media samples $3,000

12 PHA media samples $3,000

+20%

$3,000

Total $18,000

Summary and Conclusions

- Cherry and cherry products reduce plasma concentrations of inflammatory markers and the symptoms of inflammatory diseases in humans
- Inconsistencies may result from low concentration of the markers in healthy subjects, low sensitivity of the assay methods used, lack of the availability of standardized cherry based products, other dietary factors, duration of the treatment etc

Future Directions

- Develop standardized long lasting cherry products for intervention studies (canned/frozen cherries, juice, powder, extracts, food products)
- Intervention studies in people at risk (arthritis, metabolic syndrome, others)
- Dose response of cherries or cherry products
- Multiple markers, gene or RTPCR arrays, RBM

Collaborators

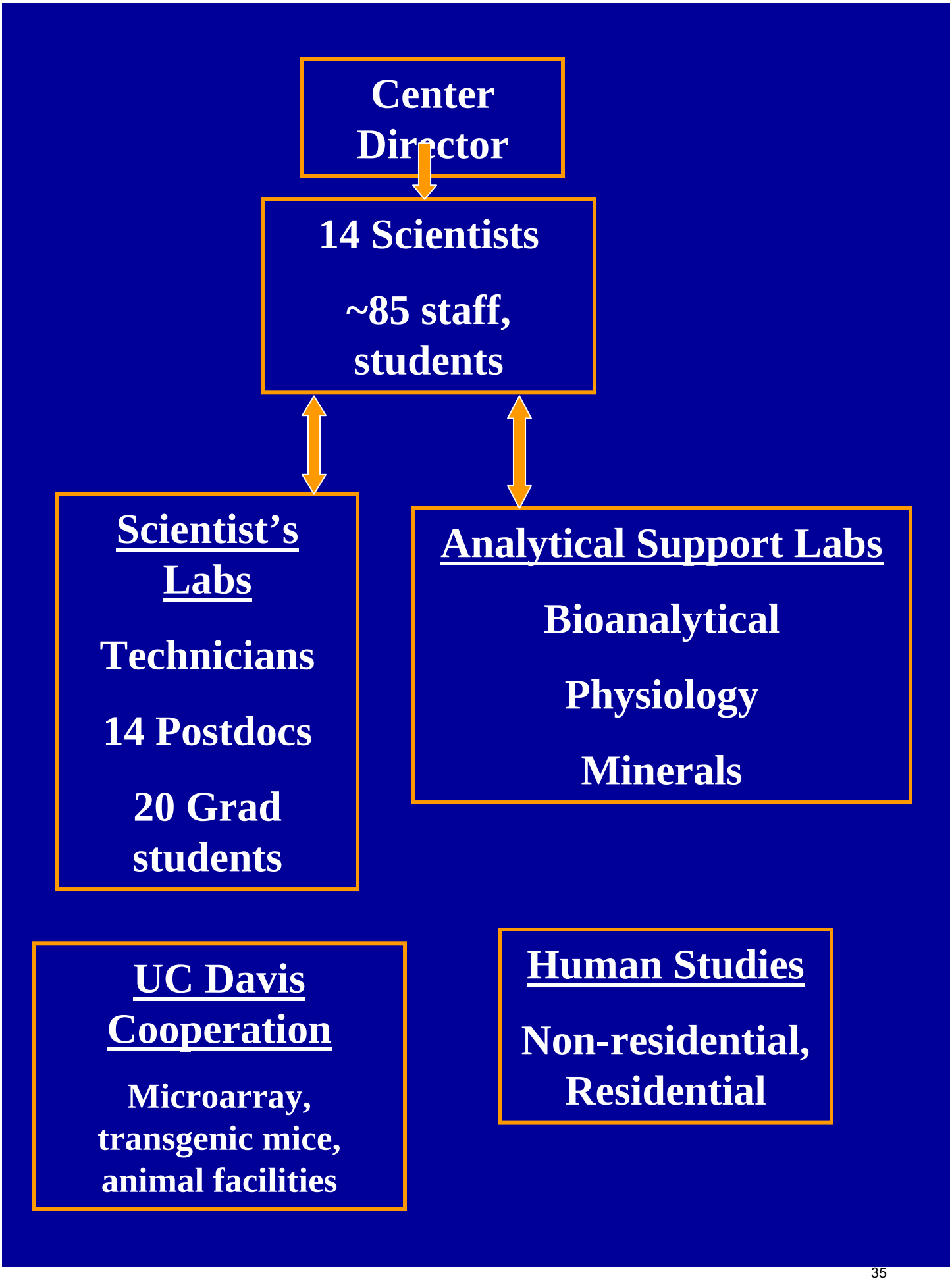
- California Cherry Advisory Board
- Robert A. Jacob, PhD, WHNRC
- Adel A. Kader, PhD, UCD
- Bruce E. Mackey, PhD, WRRC
- Yuriko Adkins, PhD, WHNRC

Foods for Health: Examples of how WHNRC studies their effects on humans

Lindsay H. Allen, Ph.D.,Director,
USDA-ARS

Western Human Nutrition Research Center

UC Davis, California


Center Director
14 Scientists
~85 staff, students
Scientist's Labs
Technicians
14 Postdocs
20 Grad students
Analytical Support Labs
Bioanalytical
Physiology
Minerals
UC Davis Cooperation
Microarray, transgenic mice, animal facilities
Human Studies
Non-residential, Residential

Research Interests

Metabolism & Obesity

- Obesity/energy (Keim)
- Metabolomics (Newman)
- Obesity/metabolism (Adams)
- Behavior (Laugero)
- Body comp. (Van Loan)
- Epidemiology (future)
- Micronutrients (Allen)
- (C-1 metabolism) (future)

Obesity

Immunity, cancer, inflam m.

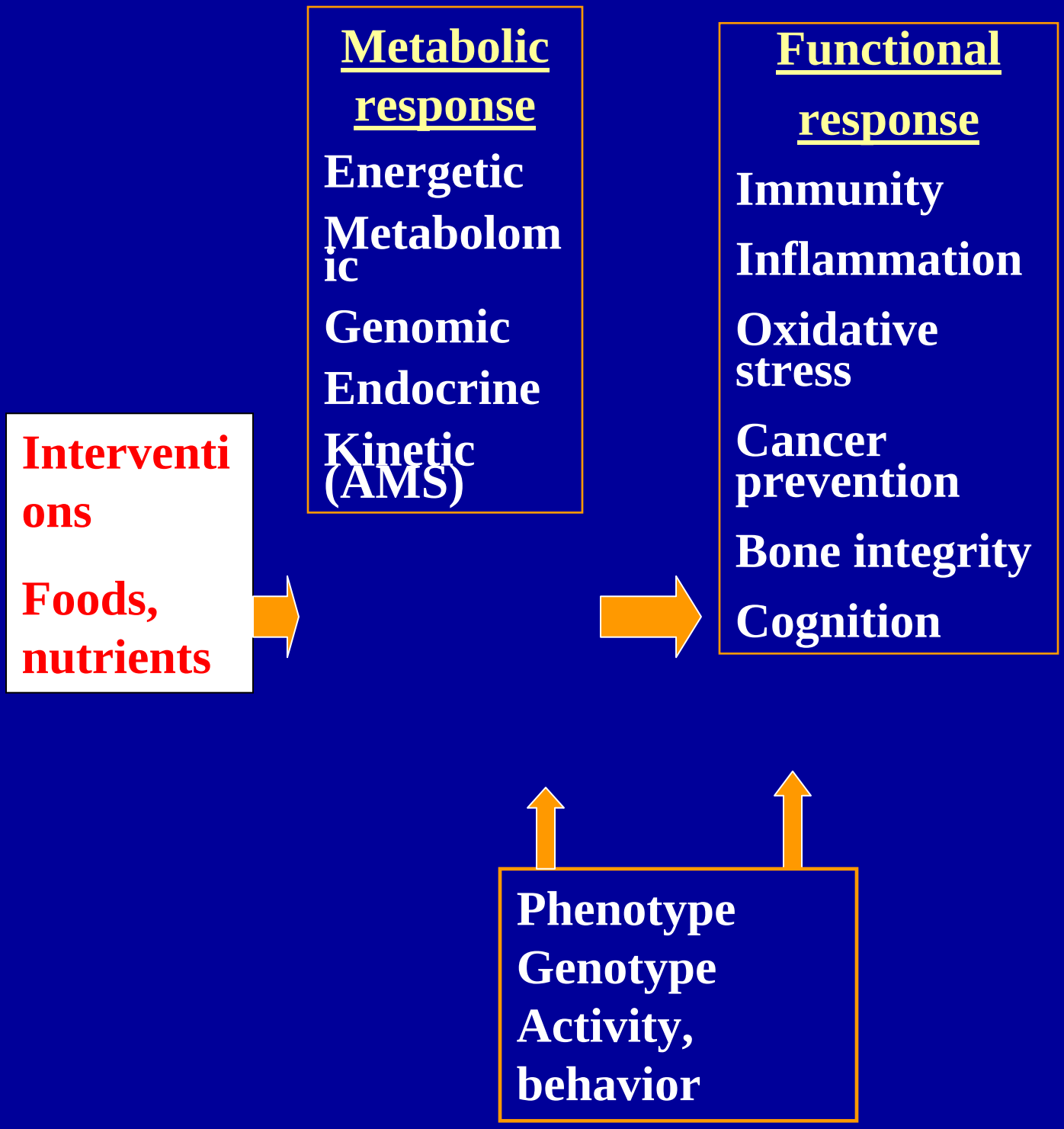
Cancer

Mineral & vitamin fx.

Nutrition, Immunity & Disease Prevntn

- Vit A,D (Stephensen)
- Phytonutr. (Zunino)
- EFA, TLR (Hwang)
- EFA, phytonut (Kelley)
- Carotene (Burri)
- Se (Hawkes)
- Zn (Huang)

WHNRC measures impact of nutrition interventions



Draft October 12, 2008

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.

By- Darshan S. Kelley, Western Human Nutrition Research Center, ARS, USDA and UCD, Davis, CA 95616. Phone # 530-752-5138; e-mail Darshan.kelley@ars.usda.gov

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 -80^{o}C 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.

Cherries, postprandial metabolism and type 2 diabetes mellitus.

PI- Arpita Basu, PhD, RD, Nutritional Sciences, OSU, OK

Co-PI- Timothy Lyons, MD, FRCP, Oklahoma Diabetes Center & Medicine Endocrinology, OUHSC, OK

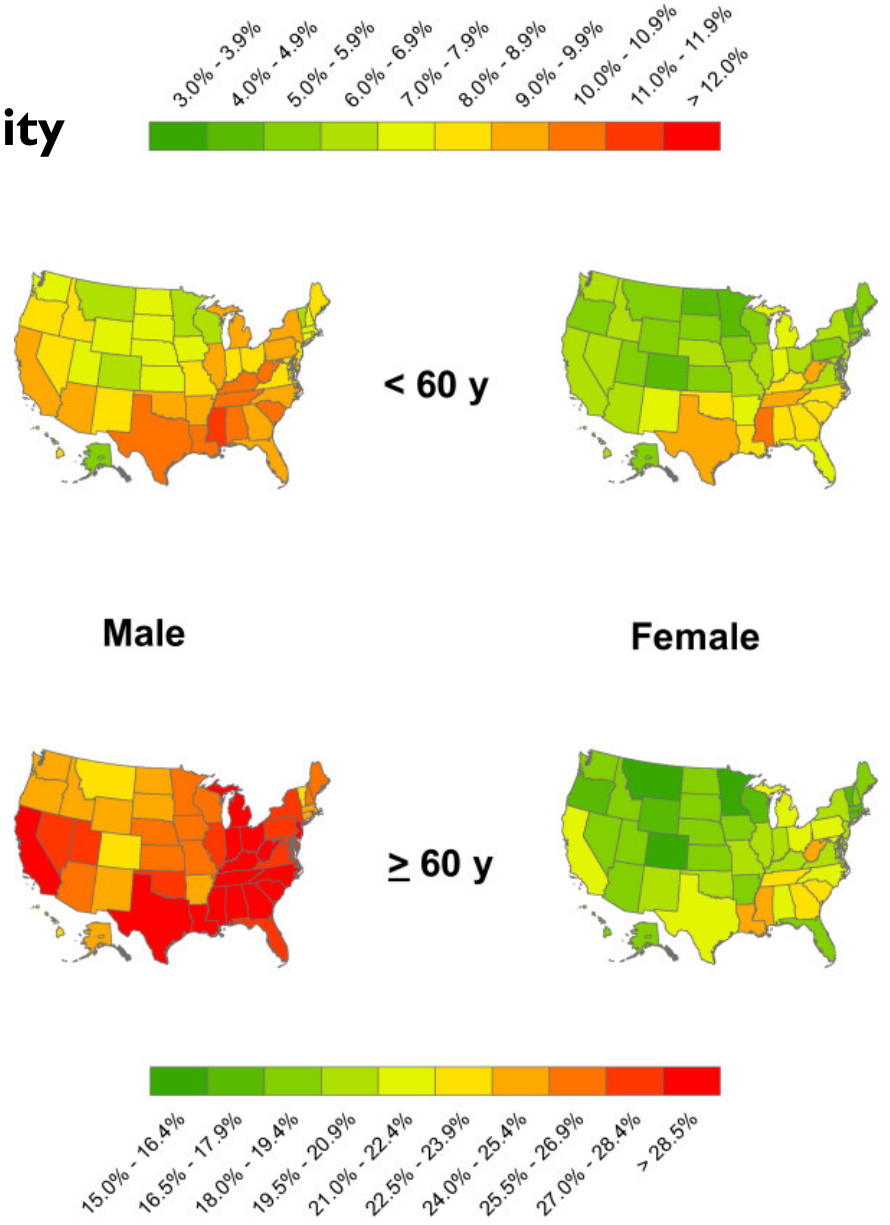
Co-PI- Chris Aston, PhD, General Clinical Research Center, OUHSC, OK

Harold Hamm Oklahoma Diabetes Center℠
THE UNIVERSITY OF OKLAHOMA

OKLAHOMA STATE UNIVERSITY

Diabetes Mellitus !

6th leading cause of mortality In the US



Oklahoma- 10.2%
US- 8.0%
(Adults >18 years)

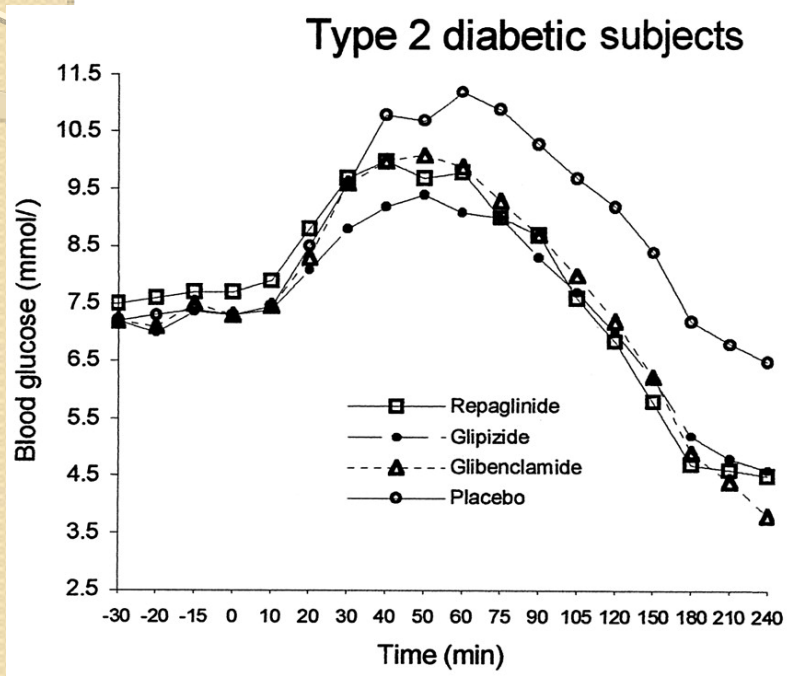
cdc.gov, Population Health Metrics, 2009

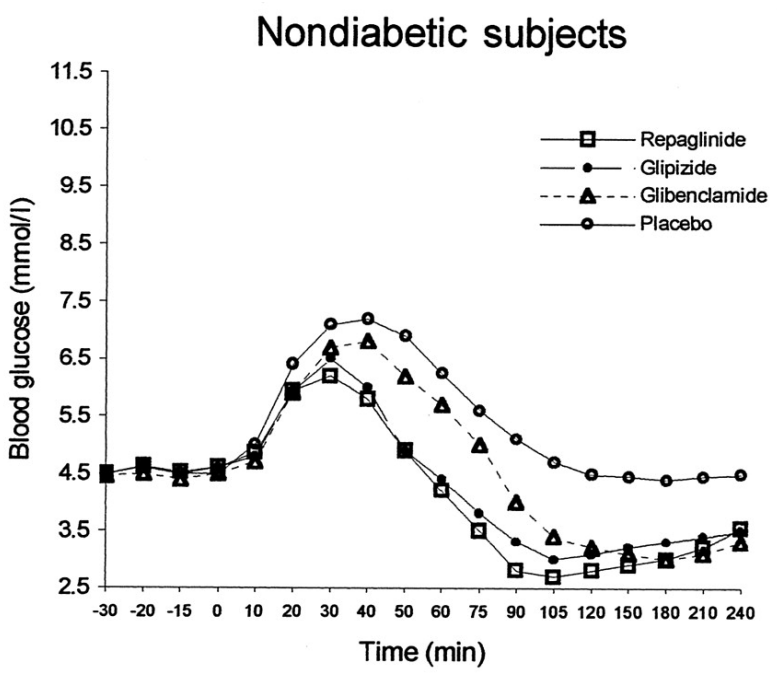
Postprandial metabolism

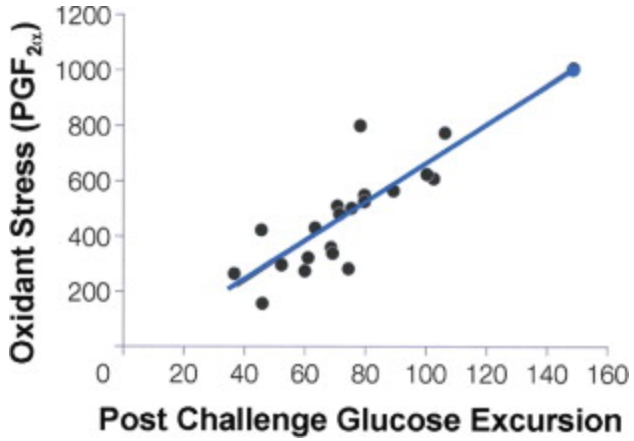
- Postprandial spikes in glucose and lipids can generate excess free radicals leading to inflammation, endothelial dysfunction and coronary artery disease

- Diets that include large amounts of fresh unprocessed fruits and vegetables, rich in antioxidants improve postprandial glucose and lipid levels
- (O'Keefe et al. J Am Coll Cardiol, 2008)

Postprandial metabolism-glucose & lipids







(O'Keefe et al. J Am Coll Cardiol, 2008)

Diet & Postprandial metabolism

Improves	Worsens
Low–glycemic index fruits and vegetables (broccoli, spinach, berries, *cherries*, tomatoes, avocados, etc.)	**Processed carbohydrates (sugar, white flour, mashed potatoes, etc.)**
Lean protein (egg whites, whey protein, fish, nonfat dairy, chicken breast, etc.)	**Saturated fat (fatty meats, full-fat dairy, etc.)**
Omega-3 (fish, nuts, etc.)	**Trans fats (french fries, commercial baked goods)**
1 or 2 alcoholic drinks/day	**Excess calories**
Fasting	

(O'Keefe & Bell, Am J Cardiol, 2007)

Phytochemicals or *Phytonutrients*

- Plant-derived compounds that have specific physiological functions beyond basic nutrition; such as, decreasing risk factors for CVD, T2DM, Cancer, etc.
  - Also classified as dietary bioactive compounds
- Several classes (2 main categories):
  - Flavonoids- catechins, anthocyanidins, pro-anthocyanidins, quercetin, etc.
  - Phenolic acids- ellagic acid, gallic acid, etc.
  - Widely present in fruits and vegetables

Why Cherries?

- Sweet cherries are high in anthocyanins and phenolic acids which have antioxidative and anti-inflammatory effects
- Low in carbohydrates and calories
  - Suitable for patients with glucose and lipid abnormalities and may improve postprandial dysmetabolism
- *No scientific data reported on the effects of sweet cherry supplementation on postprandial metabolism in patients with type 2 diabetes mellitus*

Research Aims

- **Aim 1**- To investigate the effects of sweet cherry intake on postprandial rise of glucose, insulin, lipids (total cholesterol, HDL-, LDL-cholesterol, triglycerides), and lipid peroxidation (malondialdehyde) in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

- **Aim 2**- To investigate the effects of sweet cherry intake on postprandial rise of biomarkers of inflammation (C-reactive protein, Interleukin-6, Interleukin-1β) in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

- **Aim 3-** To investigate the effects of sweet cherry intake on postprandial changes in systolic and diastolic blood pressure in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

Research design

Patients identified with abdominal adiposity and type 2 diabetes mellitus

↓

Screening visit (check safety parameters, glucose, lipids)

↓

Randomized crossover controlled design (n=20/group)

↓ ↓

LP breakfast + sweet cherry beverage **LP breakfast + control beverage**

↓

2-day intervention phase (crossover) separated by 1 week

↓

Blood pressure and blood draws at fasting, 1, 2, 4, & 6 hr each day

↓

No food or drinks except water during the 6 hour postprandial phase

↓

Subjects compensated $30/blood draw

Inclusion & Exclusion criteria

Inclusion criteria:

- Enlarged waist circumference indicative of abdominal adiposity (men >40 inches, women >35 inches)
- Type 2 diabetes mellitus on stable medications (> 1 year)
- Normal liver, kidney, and thyroid function tests

Exclusion criteria:

- Any form of pre-existing disease, e.g. cancer, heart disease, uncontrolled diabetes (fasting glucose ≥126mg/dL), on insulin therapy, hypolipidemic agents (statins)
- Liver, or renal disorders, anemia
- Pregnancy and lactation
- Taking mega doses of antioxidants/fish oil supplements (> 1g/day)
- Abnormal Hb (normal range: 12.0-18.0 g/dL), WBC (normal range: 4.0-11.0 K/mm^3), or platelets (140-440 K/mm^3), hypo/hyperthyroidism (normal range for thyroid stimulating hormone: 0.35- 4.940 uiu/mL), abnormal liver enzymes (normal range for AST: 7-40 units/L; ALT- 10-45 units/L), abnormal kidney function (normal creatinine: females- 0.7-1.2mg/dL; males- 0.8-1.2 mg/dL; normal BUN: 1-59 years- 7-18mg/dL; > 59 years- 8-21 mg/dL)
- Smoking and regular alcohol users
- Subjects having aversion or being allergic to any item in the breakfast meal, cherry and control beverages

Intervention

- High-fat low-polyphenol, low-fiber breakfast + <u>sweet cherry beverage</u>
  - 2 cups frozen, pitted sweet red cherries +1 cup water + 1 tsp vanilla essence + 1 tsp splenda
  - 120 kcal and 4g fiber

or

- High-fat low-polyphenol, low-fiber breakfast + <u>control beverage</u>
  - 2 cups water + 6 tsp sugar + 1 tsp fiber (Cellulose + Metamucil) +1 tsp vanilla essence + 1 tsp splenda
  - 120 kcal and 4g fiber

Proposed Biomarkers of analyses

- Glucose, Insulin, Insulin resistance
- Lipids (triglycerides, total cholesterol, LDL-, HDL, VLDL-cholesterol)
- Malondialdehyde (MDA)
- C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-1β (IL-1β)
- Safety parameters- liver, kidney, thyroid function tests, complete blood count (CBC)

Potential outcomes!

- Improved postprandial glucose and lipids with sweet cherry beverage intake vs. control beverage
- Decreased postprandial lipid peroxidation and inflammation with sweet cherry beverage intake vs. control beverage
- Improved CVD risk factors with sweet cherries

Limitations

- Time frame
  - Institutional Review Board (IRB)
  - Drop-outs
  - Non-compliance
  - Inclusion and exclusion criteria

- Biomarker non-detectable in plasma
  - Analysis of alternate biomarker

Budget!

- Subject compensation
- Nursing
- Bionutrition
- Laboratory costs and kits for analyses
- Biostatistician fees
- Year 1 $24,700
- Year 2 $27,200

- Thank You!
- Questions?

Sweet Cherries and Health

Cynthia A Thomson, PhD, RD
Associate Professor
Nutritional Sciences/
Arizona Cancer Center
University of Arizona
cthomson@u.arizona.edu

Objectives of SAB Discussion

- Dr. Darshan Kelley, Dr. Cheryl Rock, Dr. Andrew Breska
- Review Rules Based Medicine Study
  - Funding supported by SAB
- Review proposed feeding study in patients with diabetes mellitus
  - Merit
  - Cost:benefit ratio

  ¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤¤
- What can be extrapolated from existing research from TART cherry studies?
- When research results are available, how should they be disseminated?
  - Impact
  - legal and ethical considerations

Proposed Diabetes Study

- **Cherries, postprandial metabolism and type 2 diabetes mellitus.**
- PI- Arpita Basu, PhD, RD, Nutritional Sciences, OSU, OK
- **Aim 1-** To investigate the effects of sweet cherry intake on postprandial rise of glucose, insulin, lipids (total cholesterol, HDL-, LDL-cholesterol, triglycerides), and lipid peroxidation (malondialdehyde) in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design
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↓

2-day intervention phase (crossover) separated by 1 week

↓

Blood pressure and blood draws at fasting, 1, 2, 4, & 6 hr each day

↓

No food or drinks except water during the 6 hour postprandial phase

↓

Subjects compensated $30/blood draw

Strengths and Weakness

- Strengths:
  - Targets type II DM –common condition
  - Lowered cost with use of CTSA
  - Cherry juice: limited data
  - Targets biomarkers generally shown to be responsive in other cherry feeding trials
  - Experienced research group

Weakness: Cherry Juice Feeding Study in Type II DM

- Small sample size (n= 20)
- Cherry juice not fresh cherries; placebo juice selection
- Exclude those taking aspirin/anti-inflammatory meds?
- To some extent this replicates published work in glycemic index (pp glucose) as well as 2006 study by Kelley
- Won't move lipids in clinically meaningful way with 2 d intervention
- Time to completion (10 patients/yr)
- Short-term feeding: replicates 2006 study to some degree

Discussion /Suggestions

- Is there a cherry juice product formulated for research study?
  - Standardized product
  - Known nutrient/bioactive profile
- Is cherry juice or other cherry product an interest for focus of research or is the interest only for whole, fresh cherries?
- Study populations: disease-specific, more/less common disease, disease with common mechanisms (inflammation, oxidative stress, etc)
- Mechanistic: broader potential application, best biomarkers?, anti-inflammatory med use, baseline elevated state
- Consider more classic PK study with plasma/urine measures to assess “exposure” biomarkers and mechanistic biomarkers

Cherries: Nutritional Highlights

- Low calorie food
- Low fat food
- Low glycemic index food
- Good source of potassium
- Good source of carotenoids
- ALSO, good source of anthocyanin
- Polyphenols
- Quercetin and other bioactive food compounds

Cherry Research

Sweet

Anthocyanins:

- Cancer cell growth arrest, antioxidant, cellular differentiation, lipoxygenase inhibitors, etc
- CVD: protects against lipid peroxidation
- DM: insulin production, cherry-low GI food
- Inflammation: COX inhibition

Fresh cherry:

- Gout: decreased serum urate
- Kelley study: CRP

Tart

- As noted for anthocyanins

Also:

- Pain control in rats (cherry)
- Cecal tumor inhibition in mice (cherry extract and BAFC)
- Reduced abdominal adiposity, lipidemia, inflammation (TNFα, IL-6), PPARα and γ, MetS in rats (tart cherry powder)
- Reduced oxidative stress (8OHdG not 8-epiPGF2α) in health adults (tart cherry juice)

Focusing Research Directions

- Intervention product
  - Fresh cherries vs juice vs powder/ dietary supplement
  - Goal of this effort?
    - To sell more fresh cherry product?
    - To develop new delivery modes?
    - To target sales to “at-risk” individuals at higher cost?
    - To differentiate sour from sweet cherries?
    - All of the above? <None of the above?>

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

Argues against juice products unless skin can be compositionally included in product

Focusing Research Directions

- One cherry variety over another
- If scientific evidence suggests one variety may be favorable over another for modifying disease risk can industry “shift” to greater production? Or is it best to study a variety of commonly consumed/cultivated products?
- Rainer/Gold for COX inhibition
- Rainer, Black Gold for lipid peroxidation

Tart vs Sweet Cherry or Cherries and Health

- Benefit from simple messaging: Cherries promote health
- Anthocyanin content > in sweet (generally) than tart suggesting greater health promoting effects
- Intake of sweet cherries greater, particularly per consumption event, suggesting greater health promoting opportunity
- Tart cherry research ≠ sweet cherry
  - Relationships across industry?
  - Reality: not the same exposure effecting health outcomes
- Anthocyanin research could be applicable, but caution not to overstate

Marketing

- Caution: labeling
- Internet materials, reporting
  - "Sweet cherries are rich in anthocyanins, compounds which have been shown to XXX"
  - "Tart cherries have been shown to XXX, this has been attributed to XXX, sweet and tart cherries are generally comparable in XXX, however, this research has not been directly done in XXX subjects consuming SWEET cherries
  - Must be well qualified (in detail)
  - Structure-function claims: DSHEA
  - Do not make health claims / clinical claims

COX inhibition-
Seeram NP, *Phytomedicine*, 2001

- HPLC– anthocyanin 1 is more concentrated in tart cherries and anthocyanin 2 in sweet cherries
- Antioxidant activity 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/ratio
- Suggests sweet > tart in terms of anti-inflammatory effects

Tart Cherry Juice and Muscle Damage

- Human Performance Lab –Univ. 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
- Would not want to use: why? Small sample, subjective outcome measures, juice vs marketing fresh fruit.

Connolly DAJ, McHugh MP, Padilla-Zakour OI. BJSM 2006;40:679-83.

Current Research Needs to Advance the Health Messaging for Cherries

While human studies may be more feasible for translation more basic research is also needed to optimize study design

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

- Epidemiological studies / dietary measurement
  - Instruments lack specificity; seasonality of intake
  - Need biomarkers of intake
  - Need more specific exposure estimates: PK studies

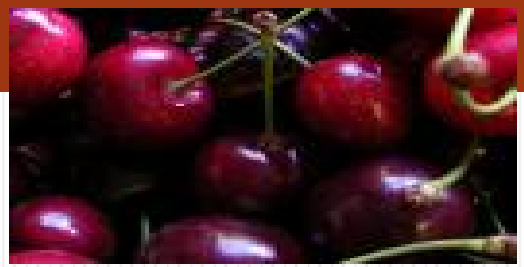
- Collaborations: multidisciplinary, translational
- Dissemination efforts – translating science for the public

Developing the Evidence Base for Health Messaging: Next Steps

- Research programming
  - Small grants
  - Research symposia
  - Peer-reviewed publications
- Scientific Advisory Board
  - Expand to include RD spokesperson? Health Educator?
  - Rotating members?
  - Translational composition

Discussion

Sweet Cherries and Health



Cynthia A Thomson, PhD, RD
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Arizona Cancer Center
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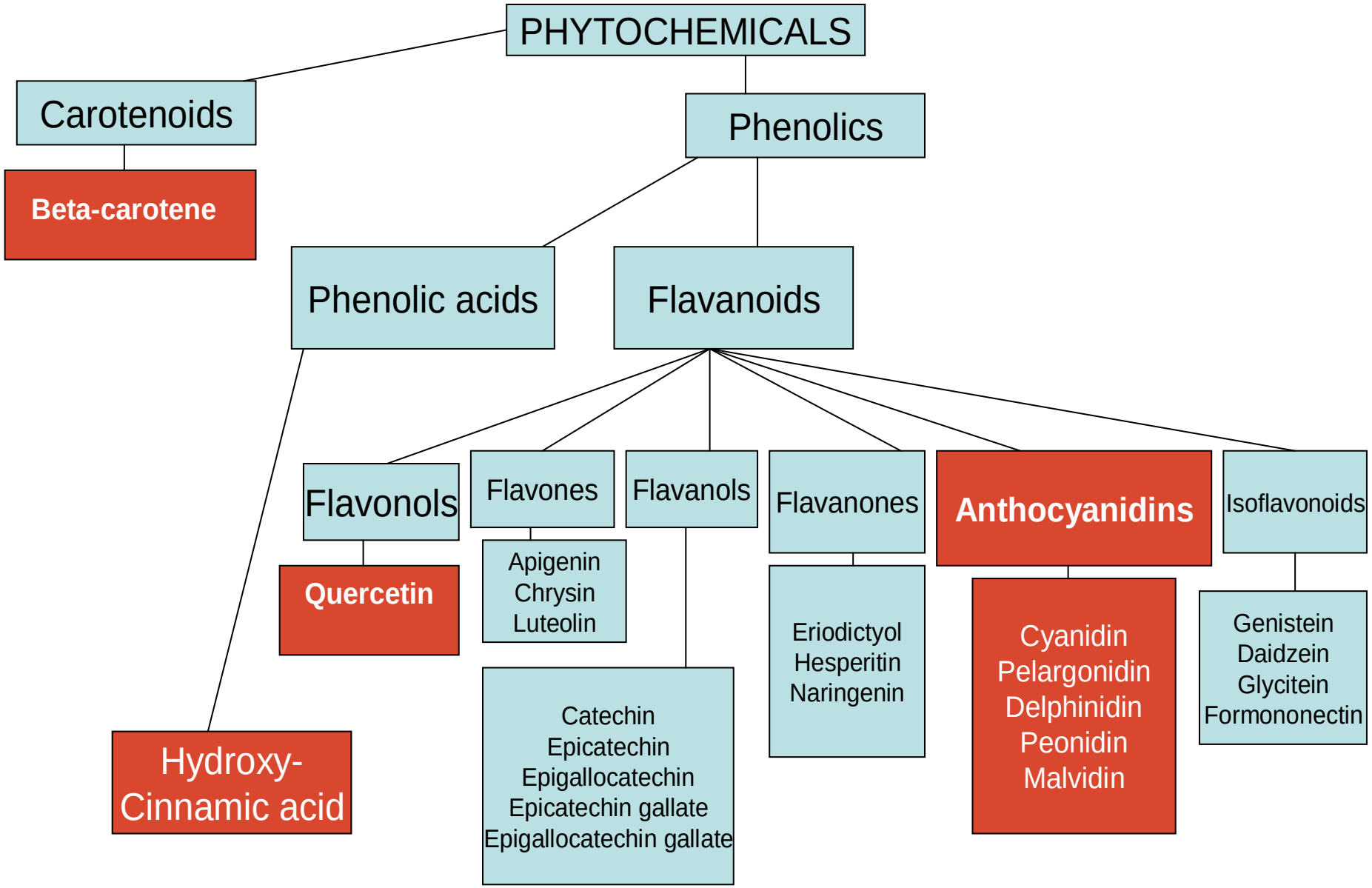
Cherries: Nutritional Highlights

- 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

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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).

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	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
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	Pits	0.1±0.0	0.54±0.04	3.38±0.26	2.00±0.13
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	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:

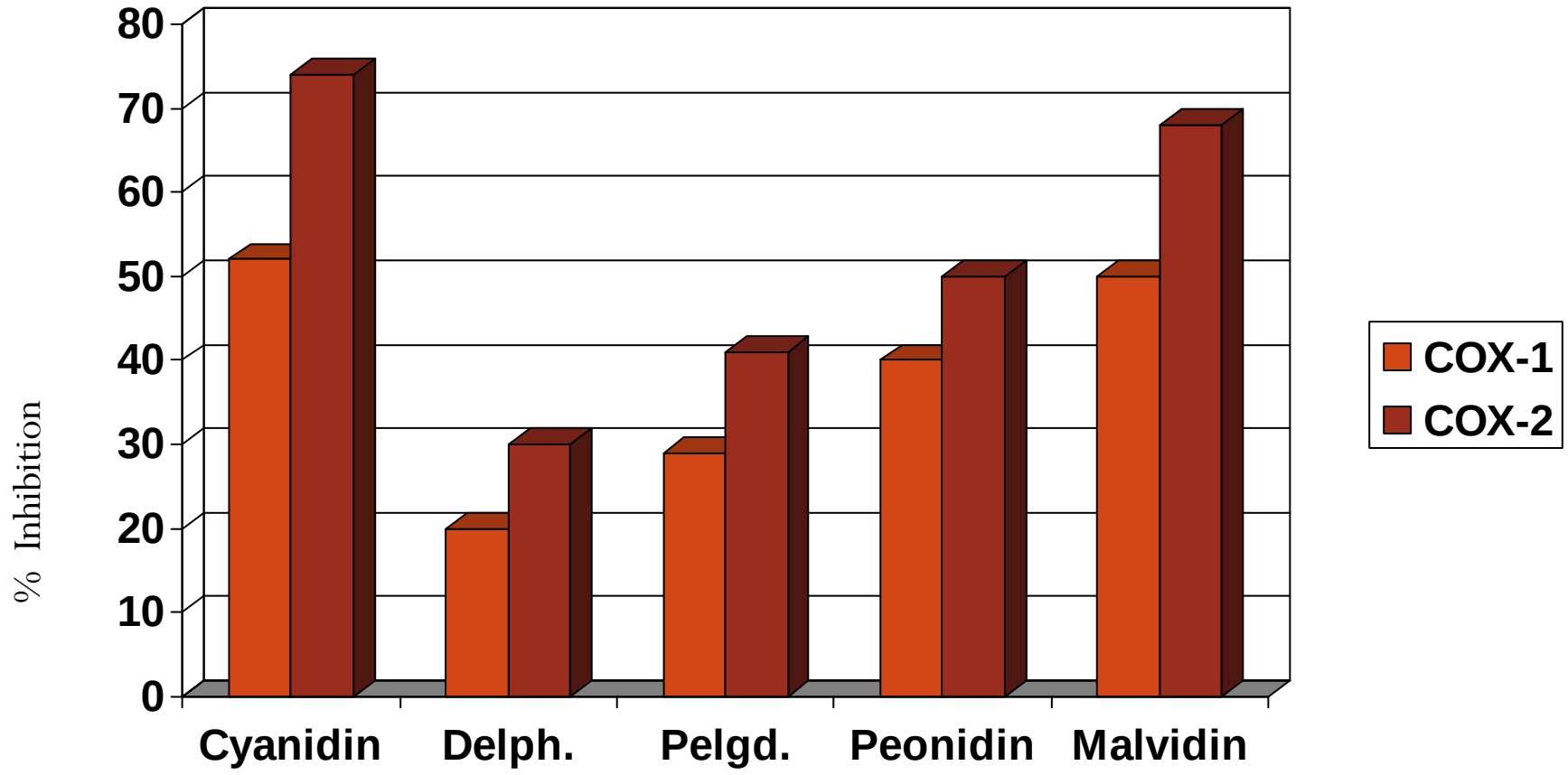
- Cancer
- Cardiovascular
- Diabetes / insulin resistance
- Arthritis
- Gout
- Alzheimers Disease

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

Andrew Willis
Northwest Cherry Growers
(509) 453-4837

Cherries and Health: A Review

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[2]Department of Plant Sciences, University of Arizona, Tucson AZ 85721

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).

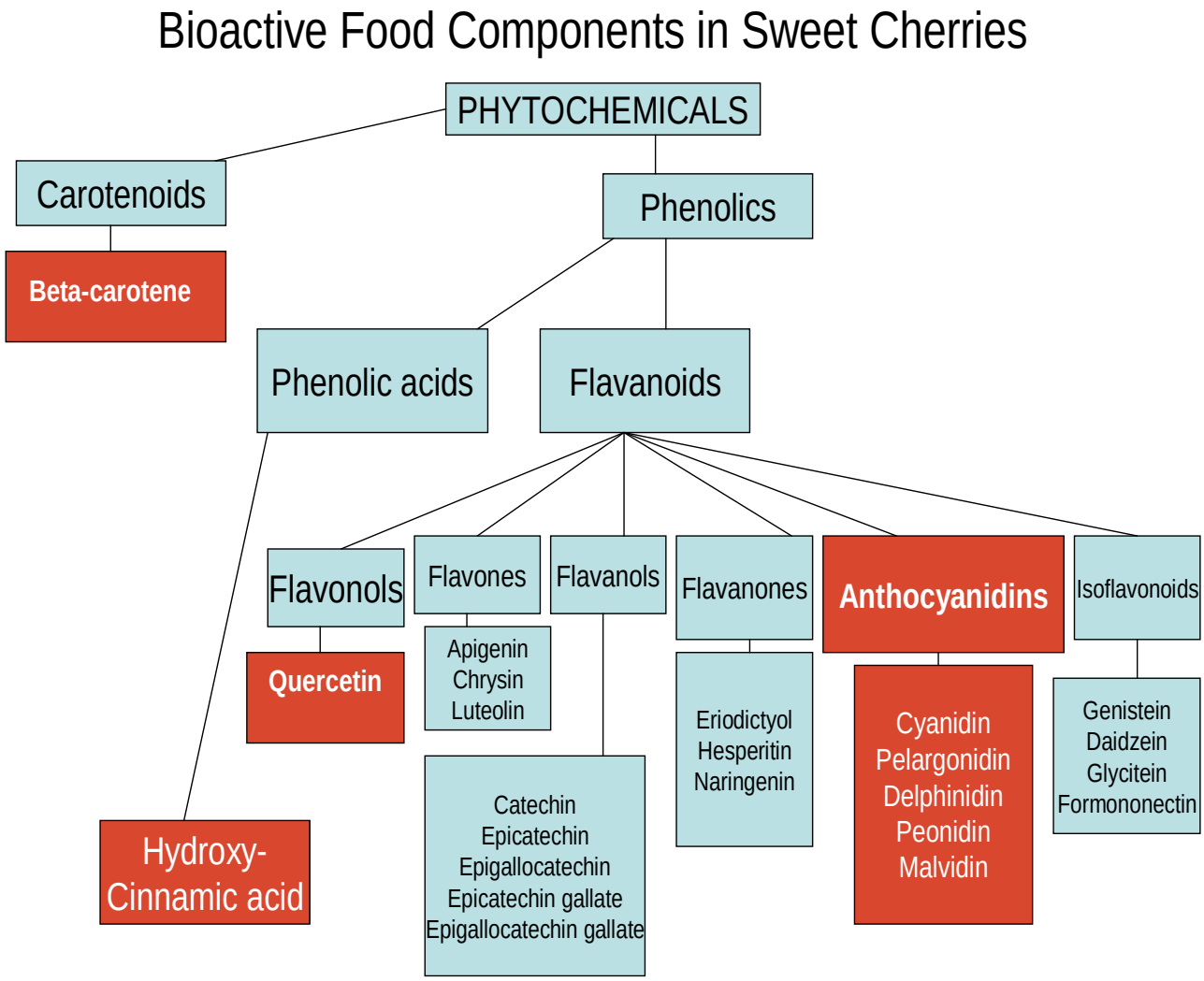
Cultivar	Portion	Anthocyanins (mg/100g fw)[Z]	Total phenolics (mg/ g fw)[Y]	ORAC (µmol TE/g fw)	FRAP (µmol TE/g fw)
Bing (sweet)	Flesh	26.0±0.7	1.34±0.18	9.07±0.35	7.28±0.24
	Pits	10.4±3.1	0.92±0.09	5.94±0.91	5.04±0.96
	Skins	60.6±2.5	3.33±0.41	28.26±1.10	21.05±0.55
Rainier (sweet)	Flesh	0.0±0.0	0.65±0.05	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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http://arthritis.about.com/b/a/200605.htm

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 2 years

Project Title: Cherries, postprandial metabolism and type 2 diabetes mellitus.

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Telephone/email: 405-271-4272/ chris-aston@ouhsc.edu
Address: 1122 NE 13th Street
City: Oklahoma City
State/Zip: OK 73117

Total Project Request: Year 1: $ 24,700 Year 2: $27,200

Budget 1
Organization Name: Oklahoma State University Contract Administrator: Becky Schlais
Telephone: 405-744-8558 Email address: becky.schlais@okstate.edu

Item	2010	2011	
Salaries (Biostatistician)	1000	1000	
Benefits			
Wages			
Benefits			
Equipment			
Supplies	2,500	5000	
Travel			
Miscellaneous			
GCRC Nursing services	5600	5600	
GCRC Nutrition services	5600	5600	
Subject compensation	4000	4000	
Laboratory costs at OU Medical Center	6000	6000	
Total	24,700	27,200	

Footnotes:

1. **GCRC Biostatistician, Dr. Christopher Aston will be conducting randomization and statistical analyses of the clinical data of patients (n=10 patients/year for 2 years)**
2. **Supplies include snack and meal components involved in the intervention, and laboratory supplies to measure markers of inflammation and lipid peroxidation (kits @ $375; 5 kits= $1875 +$625= $2,500 for year 1); increased by $2,500 in year 2 to measure markers of inflammation which can only be analyzed at the end of the study (kits @ $500; 5 kits= $2,500 added to year 2)**
3. **Nursing services have been calculated on the basis of 80 hours/year @ $70/hour= $5,600/year; nursing services will cover postprandial blood draws, processing of blood samples, monitoring patients, conducting health history examination, and reviewing medical reports with the study Co-PI (Dr. Lyons)**
4. **Nutrition services have been calculated on the basis of 80 hours/year @ $70/hour= $5,600/year; nutrition services will include conducting baseline dietary analyses for patients, preparation and administration of test meals, monitoring patients and providing specific dietary advice for enrolled patients and ensuring patient compliance**
5. **Subject compensation involves $30/blood draw for enrolled patients + additional screens**
6. **Laboratory costs at OU Medical Center will involve lab tests for fasting and postprandial glucose, lipids (total cholesterol, HDL-, LDL-cholesterol, triglycerides), insulin, and safety parameters at screen and postprandial visits +additional screens ($50/test * 11 tests/patient * 20 patients + additional screens= $ 6,000/year)**

Project Proposal

Justification:

Research has shown inadequate fresh fruit consumption by US population and a significant increase in obesity, type 2 diabetes and related cardiovascular complications. Thus, there is a pressing need for nutrition research on the health benefits of fruits and vegetables to motivate growers, researchers and the public in areas of production, scientific health claims, and consumption, respectively.

Why Cherries? A comparison among conventional fruits.

Cherries are high in phytochemicals, such as, anthocyanins and hydroxycinnamic acid, shown to be potent antioxidants and anti-inflammatory agents. ***Unfortunately, not enough research has been conducted in humans reporting the cardio protective effects of cherries. Cherries are also high in fiber and low in calories which make them better suited for patients with glucose abnormalities such as in type 2 diabetes mellitus. Furthermore, in comparison to other fruits, (Table 1) cherries provide vitamins and phytonutrients to prevent or reverse age-related oxidative damage in patients with cardiovascular risk factors, such as type 2 diabetes mellitus.***

Table 1

Fruit	Calories (kcal)	Fiber (g)	Carbohydrates (g)	Phytonutrients (mg/100g)
Cherries, Sweet, raw (1/2 cup)	**46**	**2**	**12**	**75 (cyanidin)**
Apples, raw medium, w/peel (1)	**72**	**3**	**19**	**1-7**
Banana, fresh whole, w/o peel (1)	**105**	**3**	**27**	**Negligible**
Orange, raw (1)	**62**	**3**	**15**	**1-27**

(Understanding Nutrition, Whitney & Rolfes, 2008, USDA database for flavonoid content, Release 2, August, 2006)

<u>Following is the comparison in the phytochemical content between sweet and sour cherries as listed in the USDA Flavonoid database:</u>

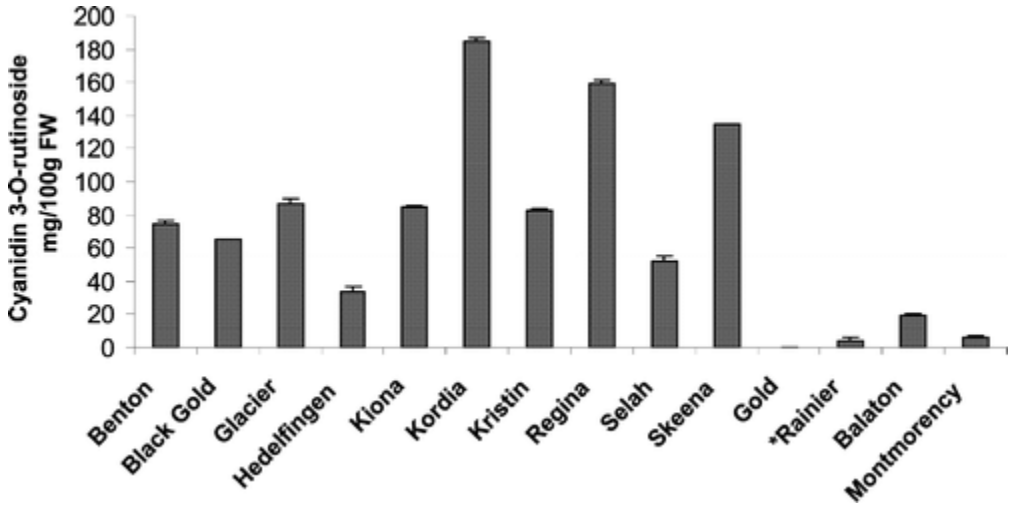
Table 2 Values (mg/100g)

Cherry	Cyanidin	Peonidin	Epicatechin	Quercetin
Sweet, raw *(Prunus avium)*	**75.18**	**4.47**	**6.97**	**2.64**
Sour, red, raw *(Prunus cerasus)*	**6.64**	**0**	**3.83**	**2.92**

(Release 2.1, January 2007)

While the flavonoid content of frozen sweet cherries has not been listed on the USDA Flavonoid database, Fazzari et al. have reported the bioavailability of phenolic compounds from five cultivars of frozen sweet cherries, thus indicating frozen sweet cherries, available commercially throughout the year, are a good source of phenolic compounds including anthocyanins (19).

Thus, sweet cherries have a significantly higher amount of anthocyanidin content which accounts for most of the antioxidant, anti-inflammatory, and cardio-protective benefits of cherries and other fruits. Mulabagal et al. have further reported a comparative analyses of anthocyanin content, lipid peroxidation, and anti-inflammatory activities of sweet and sour cherries. The following diagram shows higher amounts of cyanidin-3-O-rutinoside in sweet cherries (Benton, BlackGold, Glacier, Hedelfingen, Kiona, Kordia, Kristin, Regina, Selah, Skeena) versus sour cherries (Balaton, Montmorency)-

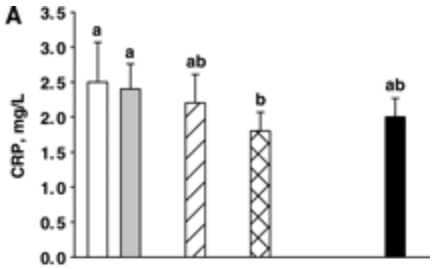


(Mulabagal et al. J Agric Food Chem, 57, 2009)

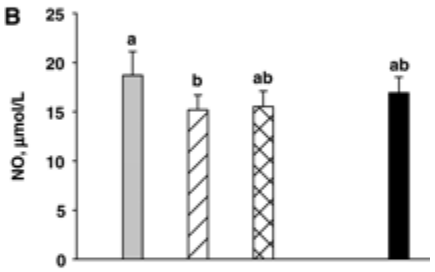
This study further showed anti-inflammatory effects of sweet and sour cherries in which the sweet cherry varieties, such as, Gold and Rainier significantly inhibited cyclooxygenase-1 & 2 enzyme activities by 81% and 94%, and 79%, and 85%, respectively, as methanol extracts. Sour cherries also inhibited these enzymatic activities in the form of water extracts. With regard to lipid peroxidation, the "Kordia" sweet cherry showed the greatest inhibition value of 88%, whereas, the sour cherries, Balaton and Montmorency, inhibited lipid peroxidation by 59% and 49%, respectively (16). Thus, sweet cherries grown primarily in the Northwest US have higher anthocyanin content, and thereby greater abilities of counteracting lipid peroxidation, and causing risk reduction of CVD than other fruits. **However, not adequate data exist on the health benefits of sweet cherries in humans with CVD risk factors, such as in type 2 diabetes, and this will be the focus of our proposed study.**

Kelley et al. (17) have reported data on the consumption of Bing sweet cherries on circulating biomarkers of inflammation in healthy men and women. While chronic inflammation is the underlying cause of the initiation and progression of many chronic diseases, including diabetes mellitus and CVD, elevated markers of inflammation are also associated with type 2 diabetes and further vascular complications of the disease. ***Thus, sweet cherry supplementation may be an effective dietary strategy for lowering inflammation at both primary and secondary level of disease***

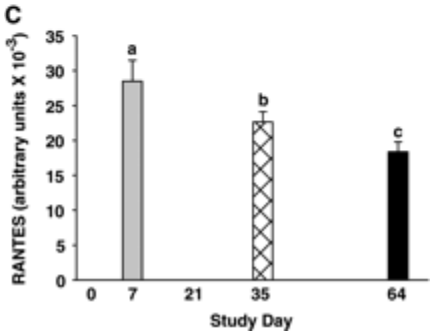
prevention. This constitutes the scope of our study in which sweet cherry supplementation on postprandial biomarkers of lipid peroxidation and inflammation in type 2 diabetic subjects will be assessed versus a control group.



C-reactive protein (CRP), a powerful predictor of CVD was shown to be reduced by 28-day supplementation of Bing sweet cherries in healthy men and women (17).



Nitric oxide (NO), a marker of inflammation and endothelial dysfunction was shown to be reduced by 28-day supplementation of Bing sweet cherries in healthy men and women (17).



RANTES, a marker of inflammation, and a surrogate marker of atherosclerosis, was reduced by 28-day supplementation of Bing sweet cherries in healthy men and women (17).

Thus, while these data look promising in reducing inflammation in healthy men and women, further studies are needed on sweet cherry supplementation in patients with higher levels of inflammation, such as in type 2 diabetes, in conjunction with the intake of a high-fat meal, and this will be addressed in our proposed study. Furthermore, as identified by Thomson & Kubota in the sweet cherry research review (18), there is a lack of human feeding trials on the cardiovascular health benefits of sweet cherries, which will be addressed by this study.

Magnitude of postprandial (after meal) rise in glucose and lipids has shown to be a significant cardiovascular risk factor in patients with diabetes mellitus. ***Plant-based dietary strategies have shown to produce a favorable impact on the rise of blood glucose and lipids, though this is an area that needs more investigation in patients. In our proposed research, we aim to investigate the effects of frozen whole cherries on the postprandial rise of glucose, lipids, and biomarkers of oxidative stress and inflammation in patients with type 2 diabetes consuming a high-fat, low polyphenol breakfast versus controls.***

Since, a typical western diet is high in fats and proteins, ***we aim to test the hypothesis that sweet cherry consumption along with a high-fat low polyphenol breakfast will decrease the rise in postprandial glucose, lipids, and markers of oxidative stress and inflammation in comparison to the control group consuming the same breakfast without cherries.*** In our study, control group will also be given a beverage containing identical amounts of fiber and calories as the cherry beverage, to elucidate the effects of cherry phytochemicals on postprandial glycemia, lipemia, oxidative stress, and inflammation.

Twenty patients with type 2 diabetes mellitus and abdominal adiposity will be randomized to the cherry or control group for the 2-day postprandial study, at least 1 week apart in a crossover design. Patients will consume a high-fat, low-polyphenol mixed meal breakfast with or without cherries. The patients will be under strict medical observation on each of the two days, when blood samples will be collected at fasting, 1, 2, 4, & 6 hours postprandial to investigate the effects of intervention on biological markers. ***In this study, we also propose to measure the classic markers of inflammation like C-reactive protein and interleukin-6 which have been associated with cardiovascular disease in diabetic patients. In addition to inflammation, we will also measure malondialdehyde, a marker of oxidative damage to lipids, to examine the extent to which cherries may prevent this lipid-associated damage in diabetic patients. Thus, in this unique study, we will address several biological markers to better explain the health effects of sweet cherries in patients with diabetes mellitus and support the inclusion of cherries as a regular fruit in their diet.***

To our knowledge, no study has been reported on the health effects of sweet cherries in patients with type 2 diabetes, a significant chronic disease in the US population. Our proposed research will address this gap and will provide scientific data for promoting sweet cherry consumption as an effective dietary therapy for postprandial dysmetabolism in these diabetic patients. This health project will also demonstrate active collaborations between the academic researchers and the cherry industry in promoting research and health awareness on specific plant-based foods. This proposed research will help in advancing knowledge about the cherries and their possible inclusion in the diet as significant sources of phytonutrients, vitamins, and fiber for the dietary management of diabetes and related cardiovascular complications.

Objectives:

Metabolic abnormalities such as type 2 diabetes mellitus and abdominal adiposity are significantly associated with impaired postprandial metabolism, increased inflammation and cardiovascular disease. The overall objective of this 2-year clinical trial is to investigate the effects of sweet cherries on postprandial glucose, lipids, insulin, lipid peroxidation and inflammation in subjects with abdominal adiposity and type 2 diabetes mellitus in a randomized crossover controlled trial. Our study objective conforms to the priorities of cardiovascular health and inflammation as identified in the nutrition research program of the Oregon Sweet Cherry Commission and the Washington Tree Fruit Research Commission. Keeping in view the high burden of type 2 diabetes and cardiovascular disease and related dietary inadequacies in Oklahoma, our primary goals of Nutritional Sciences at OSU, the General Clinical Research Center (GCRC), and the Harold Hamm Oklahoma Diabetes Center (HHODC) at OUHSC are to address these cardiovascular health issues through dietary improvements. Our clinical research interests encompass special emphasis on functional foods such as cherries high in antioxidants and other dietary bioactive compounds.

The specific aims are as follows-

Aim 1- To investigate the effects of sweet cherry intake on postprandial rise of glucose, insulin, lipids (total cholesterol, HDL-, LDL-cholesterol, triglycerides), and lipid peroxidation (malondialdehyde) in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

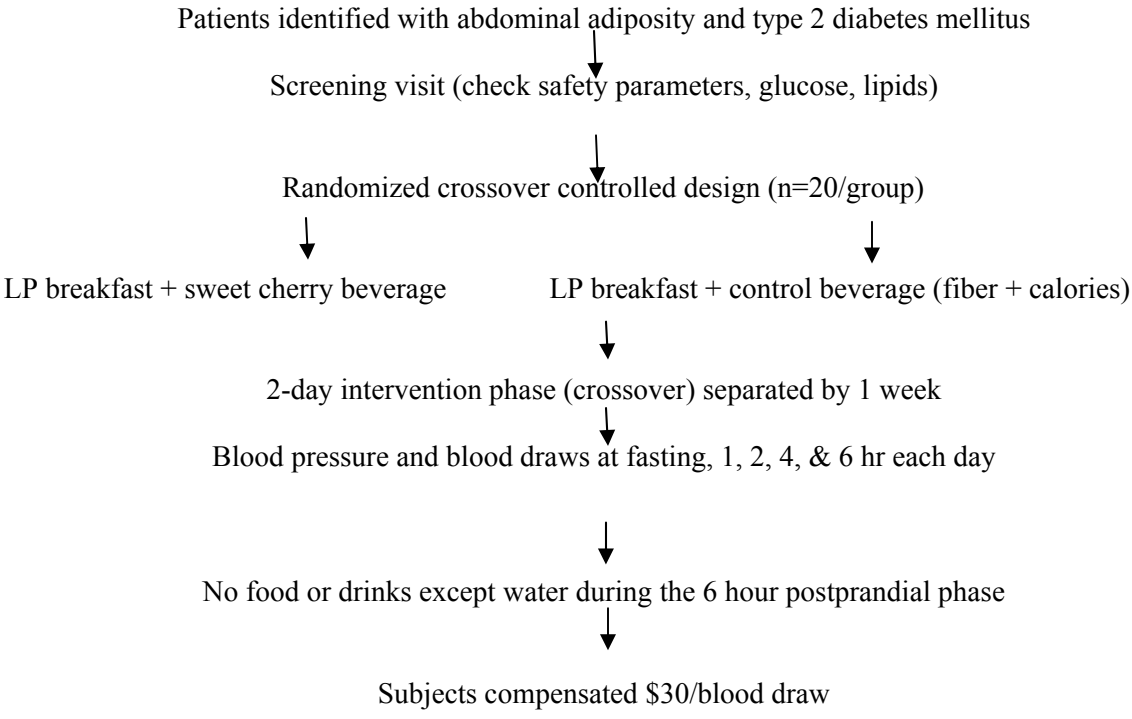
Aim 2- To investigate the effects of sweet cherry intake on postprandial rise of biomarkers of inflammation (C-reactive protein, Interleukin-6, Interleukin-1β) in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

Aim 3- To investigate the effects of sweet cherry intake on postprandial changes in systolic and diastolic blood pressure in subjects with abdominal adiposity and type 2 diabetes versus control beverage in a randomized crossover design

In addition to the above parameters, we will also conduct liver, kidney, and thyroid function tests at screen and 6 hours of each postprandial phase. Aims 1, 2, & 3 will be simultaneously conducted using fasting and postprandial blood samples drawn during two experimental visits by each patient. We propose to recruit 20 patients (10/year) over a period of 2 years to undergo this randomized controlled phase of test meals with and without sweet cherries.

Methods:

Research design



LP- Low polyphenol

Inclusion criteria: Subjects with enlarged waist circumference indicative of abdominal adiposity (men >40 inches, women >35 inches) and type 2 diabetes mellitus on stable medications (> 1 year) as defined by National Cholesterol Education Program, Adult Treatment Panel III will be included in the study. Subjects with normal liver, kidney, and thyroid function tests will be included in the study. Subjects on stable multivitamin/mineral supplements or prescription medications (except insulin therapy, and hypolipidemic agents like statins) will be included in the study. Subjects will continue their routine diabetic medications throughout the study.

Exclusion criteria: any form of pre-existing disease, e.g. cancer, heart disease, uncontrolled diabetes (fasting glucose ≥126mg/dL), on insulin therapy, hypolipidemic agents (statins), liver, or renal disorders, anemia, pregnancy and lactation, taking mega doses of antioxidants/fish oil supplements (> 1g/day), abnormal Hb (normal range: 12.0-18.0 g/dL), WBC (normal range: 4.0-11.0 K/mm^3), or platelets (140-440 K/mm^3), hypo/hyperthyroidism (normal range for thyroid stimulating hormone: 0.35- 4.940 uiu/mL), abnormal liver enzymes (normal range for AST: 7-40 units/L; ALT- 10-45 units/L), abnormal kidney function (normal creatinine: females- 0.7-1.2mg/dL; males- 0.8-1.2 mg/dL; normal BUN: 1-59 years- 7-18mg/dL; > 59 years- 8-21 mg/dL), smoking, and regular alcohol users will be excluded from the study. Both males and females, as well as individuals from any ethnic group, who qualify, will be included in the study. Subjects having aversion or being allergic to any item in the breakfast meal, sweet cherry and control beverages will be excluded from the study. This also includes subjects who are vegans and/or vegetarians.

Recruitment and Randomization: Following approval from the Institutional Review Board (IRB) at OUHSC and OSU, subjects will be recruited at the General Clinical Research Center (GCRC) at OUHSC and at the clinical assessment facilities at OSU. Following an initial telephone screen, subjects will be scheduled for a screening visit and qualification will be confirmed based on diagnosis of type 2 diabetes mellitus and specific measurements such as waist circumference and fasting glucose and lipid profile, and safety parameters. Upon qualification, patients will be randomized to the sweet cherry or control beverage with high-fat low polyphenol mixed meal breakfast, followed by crossover to the subsequent intervention at least one week later. On each day of the 2-day postprandial phases, patients will arrive at the clinic around 7:30AM for a fasting blood after which, they will consume the breakfast meal with or without cherries within 20-25 minutes, followed by postprandial blood draws at 1, 2, 4, & 6 hours. Subjects will be strictly monitored at the clinic and will be allowed to drink only water for 6 hours. Lunch will be provided at the end of six hours.

Intervention: The nutrient composition of the high-fat low polyphenol mixed meal breakfast is shown below:

Table 2

Nutrient	Amount
Kcals	**496.32**
CHO (g)	**45.71**
Protein (g)	**18.87**
Total Fat (g)	**26.89**
SFA (g)	**11.57**
MUFA (g)	**6.53**
PUFA (g)	**2.74**

Cholesterol (mg)	**474.62**
Fiber (g)	**3.41**
Vitamin C (mg)	**7.39**
Vitamin E (mg)	**1.12**

The breakfast meal will consist of the following items:

Egg- 1, Egg yolk-1, White bread- 2 slices, Butter- 13gm, Banana-1, Pork sausage, patty, cooked- 26gm; corresponding to 50% fat, 35% carbohydrate, 15% protein

Cherry beverage- Two cups frozen pitted **sweet red cherries** (purchased from local grocery store) will be thawed for 20 min at room temperature, and blended in 1 cup water, with 1 tsp vanilla essence, and 2 tsp splenda (artificial sweetener). The cherry beverage will provide 120 calories and 4g fiber

Control beverage- Two cups water will be blended with 6 tsp sugar, 1 tsp fiber (Cellulose + Metamucil), 1 tsp vanilla essence, 2 tsp splenda and 1 tsp artificial cherry flavor extract. The control beverage will provide 120 calories and 4g fiber.

The subjects will be asked to consume all items in the breakfast meal and the sweet cherry or control beverage within 20-25 minutes under observation.

Dietary analyses- Patients will be asked to maintain their usual diet and lifestyle activities while on the study, except the 2-day postprandial visits when meals will be provided by the clinic. Baseline analyses of diet will be conducted by the GCRC Bionutrition staff. Three day averages of micro and macronutrient intakes will be analyzed using Nutritionist Pro (version 3.2, 2007, Axxya Systems, Stafford, TX). All data entry will be performed by RDs at GCRC who are trained and certified in using the software. All dietary data entry will be verified by a second RD as a measure of quality control. If a participant ate a food that was not in the database, a food with very similar nutrient composition will be chosen. Nutrient information will also be obtained through food labels or recipes from subjects, online sources, or at grocery stores.

Clinical Variables: Fasting blood samples, after each draw will be immediately sent to OU Medical Center laboratory for comprehensive metabolic panel (CMP) including glucose, insulin, lipid panel (total cholesterol, LDL-, HDL-cholesterol, triglycerides), serum electrolytes, liver, kidney, thyroid tests, and complete blood count at each draw. Remaining plasma and serum samples will be stored at -80°C for subsequent analyses of biomarkers of lipid peroxidation and inflammation.

Biomarkers of oxidative stress and inflammation: Lipid peroxidation will be measured in serum as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), using a colorimetric assay according to the manufacturer's protocol (LPO-586^{TM}, Oxis Health Products, Inc., Portland, OR). Markers of inflammation (hs-CRP, IL-6, IL-1β) will be measured using ELISA kits (R&D Systems, Inc. Minneapolis, MN) according to the manufacturer's protocol. All samples will be assayed in triplicates within our established inter assay variations of 10% for each variable.

Power calculations and Statistical analyses: Based on our data from previous studies, we will have 80% power to detect 21.8% difference in triglycerides and 30.6% difference in malondialdehyde with a sample size of 40 (20/group) at a significance level of 5%. Repeated measures ANOVA with post-hoc comparisons will be used to detect changes on the variables of interests and data will be analyzed for outliers using SPSS (version 16.0).

Limitations: Completion of target recruitment within the proposed time frame is the major limitation of clinical research. However, since recruitment will be conducted at GCRC (CRC), OUHSC, and subjects will be drawn from our existing database in addition to multi-channel advertisements (campus e-mails, flyers, website headlines, physician referrals), we hope to reach the target within 2 years of the proposed recruitment phase. Our laboratory staff is well trained in clinical and experimental analyses. However, there may be instances when certain markers of oxidative stress or inflammation may be non-detectable in human plasma samples, in which case an alternative relevant marker will be measured. Every effort will be made to complete recruitment within the proposed time frame. However, we may ask for an extension of time to complete patient recruitment since in the opinion of our research group, data quality and adhering strictly to the inclusion and exclusion criteria are crucial for a sound scientific study. Thus, we will continue vigorous recruitment based on the standards of good clinical practices and make sure the aims are completed, though an extension of time may be necessary. The other factor that may delay our progress is the possibility that Institutional Review Board (IRB) may delay or postpone their meetings and as a result, the approval of this study. However, based on the successful completion of our previous clinical nutrition trials, we hope to achieve our goals based on perseverance and implementation of scientific study design and post analyses.

Expertise of PI and research group: The PI (Basu) and the Co-PI (Lyons) have conducted clinical trials with successful recruitment and compliance rates. Please visit
http://gcrc.ouhsc.edu/ProtocolListing.asp
for our collaborative projects on Green Tea and Blueberry studies. Our research group specializes in patient-oriented research in the areas of metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease. We have established a database of approximately 250 type 2 diabetic subjects which will greatly assist our recruitment efforts. We have well –trained laboratory staff in measuring biomarkers of inflammation and lipid peroxidation which will be the main variables in this study. Our study group has recently published a pilot clinical trial on strawberries showing the potential of fruit phytochemicals to affect cardiovascular risk factors (15). Thus, with our fully equipped CRC team including registered nurses, dietitians, physicians, and biostatisticians, we possess the necessary skills and resources for the successful implementation and completion of the proposed study design.

Proposed schedule of accomplishments:

Year 1- Approval from Institutional Review Board (IRB) at OUHSC, 50% target recruitment, blood draws and dietary analyses, laboratory analyses of clinical variables and oxidative stress, mid-point data analyses

Year 2- IRB approval renewal, completion of remaining 50% recruitment, blood draws and dietary analyses, laboratory analyses of clinical variables, oxidative stress and inflammation, final analyses of clinical data

Review of literature:

Fruits and vegetables high in phytochemicals have been shown to reduce the risk factors of cardiovascular disease in several observational studies (1-3). Among fruits, cherries, berries, and grapes are especially high in cardio protective nutrients or biofactors that may reverse significant CVD risk factors. Studies have shown that red sweet cherries high in anthocyanins, particularly, cyanidin-3-O-rutinoside, significantly inhibit lipid peroxidation and cyclooxygenase enzymatic activity, thereby acting as antioxidants and anti-inflammatory agents, respectively, in experimental conditions (4). Animal studies have further shown that cherry juice supplementation increased antioxidant enzymatic concentrations in liver and blood of mice, and significantly decreased lipid peroxidation (5). Cherry anthocyanins were further shown to decrease markers of inflammation like tumor necrosis factor-alpha in serum and prostaglandins E2 in paws of arthritic rat model. Data also reported antioxidant effects of cherry anthocyanins in significantly decreasing serum malondialdehyde concentrations, a biomarker of lipid peroxidation (6). Cornelian cherries have been shown to reduce risk factors of diabetes, by ameliorating weight gain and glucose intolerance in high-fat fed mice (7). Though human studies on health benefits of cherries are limited, tart cherry juice has been shown to improve antioxidant defenses in healthy older men and women (n=12) (8). However, these results need to be confirmed in larger controlled trials. ***Thus, these in vivo animal studies and limited human data provide evidence on the antioxidative and anti-inflammatory functions of cherries as potential dietary agents in ameliorating oxidative stress and inflammation underlying CVD and dysmetabolism as seen in diabetes mellitus.***

Abdominal adiposity and type 2 diabetes mellitus are associated with chronic oxidative stress and inflammation and abnormal postprandial metabolism (9, 10). Postprandial rise of triglycerides has also been significantly correlated with atherosclerosis. ***Researchers have suggested that the composition of meal has the potential to produce favorable impacts on postprandial metabolism. Human studies have shown that certain foods like almonds, vinegar, whey protein and low carbohydrate fruits like cherries and grapefruits can decrease postprandial glucose and lipids in comparison to meals without these foods (11, 12). Since, obesity, diabetes mellitus, and cardiovascular disease continue to contribute to causes of morbidity and mortality in the US, effective dietary strategies to lower risk factors need more attention in preventive health care.***

This proposed study will address the significant gap in research on the postprandial effects of low carbohydrate fruits, such as cherries, in subjects with type 2 diabetes mellitus. On the basis of previous research that has shown that fruit phytochemicals are associated with increased serum antioxidant capacity in the postprandial phase in human subjects (13, 14), there may be a possibility that cherry phytochemicals may lower lipid peroxidation and inflammation in the postprandial phase. This constitutes the focus of our proposed study and the resulting data will form the basis for effective medical nutrition therapy for patients with abdominal adiposity and type 2 diabetes mellitus.

References:

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Article

Anthocyanin Content, Lipid Peroxidation and Cyclooxygenase Enzyme Inhibitory Activities of Sweet and Sour Cherries

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Abstract

Full Text HTML

Hi-Res PDF [644 KB]

PDF w/ Links [212 KB]

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Abstract

Cherries contain bioactive anthocyanins that are reported to possess antioxidant, anti-inflammatory, anticancer, antidiabetic and antiobese properties. The present study revealed that red sweet cherries contained cyanidin-3-*O*-rutinoside as major anthocyanin (>95%). The sweet cherry cultivar "Kordia" (aka "Attika") showed the highest cyanidin-3-*O*-rutinoside content, 185 mg/100 g fresh weight. The red sweet cherries "Regina" and "Skeena" were similar to "Kordia", yielding cyanidin-3-*O*-rutinoside at 159 and 134 mg/100 g fresh weight, respectively. The yields of cyanidin-3-*O*-glucosylrutinoside and cyanidin-3-*O*-rutinoside were 57 and 19 mg/100 g fresh weight in "Balaton" and 21 and 6.2 mg/100 g fresh weight in "Montmorency", respectively, in addition to minor quantities of cyanidin-3-*O*-glucoside. The water extracts of "Kordia", "Regina", "Glacier" and "Skeena" sweet cherries gave 89, 80, 80 and 70% of lipid peroxidation (LPO) inhibition, whereas extracts of "Balaton" and "Montmorency" were in the range of 38 to 58% at 250 µg/mL. Methanol and ethyl acetate extracts of the yellow sweet cherry "Rainier" containing ß-carotene, ursolic, coumaric, ferulic and cafeic acids inhibited LPO by 78 and 79%, respectively, at 250 µg/mL. In the cyclooxygenase (COX) enzyme inhibitory assay, the red sweet cherry water extracts inhibited the enzymes by 80 to 95% at 250 µg/mL. However, the methanol and ethyl acetate extracts of "Rainier" and "Gold" were the most active against COX-1 and -2 enzymes. Water extracts of "Balaton" and "Montmorency" inhibited COX-1 and -2 enzymes by 84, and 91 and 77, and 87%, respectively, at 250 µg/mL.

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COMPREHENSIVE INDUSTRY STRATEGIC PLAN (CISP)
SUMMARY OF KEY MEETINGS AND RECOMMENDATIONS

The California Cherry Advisory Board (CCAB) and the Washington State Fruit Commission (WSFC) representing sweet cherry production in Washington, Oregon, Idaho, Utah and Montana have a history of working together to increase market access and demand for sweet cherries around the world. Since 2006, CCAB and WSFC have formalized their cooperation in the development and execution of a Comprehensive Industry Strategic Plan (CISP). Now in the third year of this effort, CCAB and WSFC are working even more closely to ensure the long-term health of the sweet cherry industry.

The First Meeting
CCAB and WSFC representatives first met in March 2006 to identify where the industries already work together and opportunities for increased cooperation. The conclusions and recommendations coming out of this meeting formed the basis for the U.S. fresh sweet cherry industry's CISP. A copy of that report has been forward to USDA/FAS.

Overall, the CCAB and WSFC representatives found that the two organizations already collaborate on a number of initiatives related to production research, trade policy, marketing, and health research. However, more can be done to strengthen these ties. In particular, the CCAB and WSFC representatives at the meeting suggested the organizations should:

- Explore grant opportunities to help fund research on the possibility of conceptualizing, developing, and testing mechanical harvesting methods
- Seek university support and grant opportunities to fund research to explore the benefits and challenges of converting existing orchards to a "fruiting wall" system to aid in harvest
- Develop a long-term research plan to improve product quality and safety through chemical usage, orchard management, and varietal development
- Continue to address retailers' desire for single-source supply-managers by considering further alliances within the industry such as sales desk consolidation and packing shed management
- Make it standard operating procedure to coordinate trade policy efforts between the two organizations
- Seek partnerships with European suppliers to gain a better understanding of competitive factors in Europe and to help define windows of opportunity
- Share representatives and resources where is makes sense to do so
- Explore grant opportunities to conduct exploratory research in India, China, and Russia
- Form a Health/Nutrition Committee to develop and implement a health research plan

Since this initial meeting, the organizations have started to implement a number of the recommendations. In particular, WSFC commissioned research studies in the Ukraine and Russia to better understand: (1) Ukraine's cherry production capacity and potential competitive pressures in European markets; and (2) to determine opportunities for U.S. cherry exports to Russia and conduct a test promotion to gauge trade and consumer interest. These studies provided valuable information that industry can use to build market development efforts.

The Second Meeting

The results of these reports were shared at a second meeting between CCAB and WSFC representatives held in January 2008. In addition to reviewing the research, the organizations also discussed the health benefits of cherries and ways to better communicate those results. Discussions surrounding health research and communication consumed the bulk of the meeting agenda.

Through the course of the discussion, the organizations outlined seven key questions that industry must answer as it considers health research and communication including:

1. Should the sweet cherry industry pursue health messaging?
2. What should be the key message?
3. What can/cannot be said about sweet cherry health benefits?
4. What holes need to be filled in the scientific community's understanding of cherry health benefits?
5. What is the potential impact of pursuing a health research/communication strategy?
6. How does the industry pay for it?
7. What should CCAB/WSFC do to communicate results?

Formation of a Health & Nutrition Committee and Scientific Advisory Board

To help answer these questions and prioritize resources, CCAB and WSFC formalized a Health & Nutrition Committee (HNC) made up of select industry members from both organizations. The committee is charged with the following tasks:

- Developing a formal structure among all stake holders to manage health research and communication projects and resources
- Deciding which direction industry will take in the pursuit of sweet cherry health research
- Identifying holes in the existing research
- Systematically working to fill the knowledge gaps so that industry can speak credibly about cherry health benefits
- Creating a budget and identifying funding sources for research

The HNC sought further advice on these health matters through the formation of a Scientific Advisory Board (SAB). On March 2, 2009, the newly formed SAB meet in Seattle, WA to start answering 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?

Members of the SAB include:

Andrew Breksa
Research Chemist & Lead Scientist
USDA, ARS, WRRC

Darshan S. Kelley, Ph D
Research Chemist/Adjunct Professor
Western Human Nutrition Research Center, ARS, USDA
Department of Nutrition, University of California at Davis

Cheryl L. Rock, PhD, RD
Professor
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School of Medicine
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Cynthia Thomson PhD, RD
Consulting SAB Director
Associate Professor
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During this first meeting of the SAB, eight recommendations for future research emerged. These were prioritized based on the need to build a foundation for understanding and growing that understanding with each successive research project. However, some of these projects can be conducted at the same time if funding is available. Additional information about each of these recommendations is provided below:

1. **Rules Based Medicine Study** *Estimated Cost: $115,500*
   In 2006, the University of California at Davis and Western Human Nutrition Research Center (WHNRC) conducted a feeding study on Bing cherries to determine:
   - Serum concentration of markers of inflammation
   - Blood lipids, lipoproteins, particle size and number
   - Hematology and clinical chemistry panels including insulin

   Dr. Kelley was one of the lead researchers on this project. Before eating cherries and at regular intervals after eating the fruit, research subjects had their blood drawn. The results showed that cherry consumption had significant effects on some circulating markers of inflammation, but not all that were expected. The changes were too small to attribute to cherries.

Since this research was conducted, new methods for biomarker testing have been developed by Rules-Based Medicine (RBM), the world's leading multiplexed biomarker testing laboratory. If approved for funding, RBM under the guidance of Dr. Kelley's lab would analyze plasma and media samples collected from cultured white blood cells obtained from the previous cherry feeding study to determine the effects of the antioxidant nutrients in fresh sweet cherries on biomarkers tied to the prevention and reversal of chronic inflammatory diseases including cardiovascular disease, insulin resistance, diabetes, immune status, and cancer.

More specifically, RBM will analyze plasma and media samples using two MAP assays. The first assay, (Human MAP Version 1.6) will analyze the plasma samples for 89 antigens that include pro-and anti-inflammatory cytokines, growth factors, adhesion molecules, clotting factors, hormones, and markers for immune status including allergies, and cancer. The second assay (Human MAP Version 1.1) will analyze the media samples for 46 antigens with a focus on pro-and anti-inflammatory factors. The output of RBM's MAP assays will form the basis for the findings in this study that will be analyzed by the researchers at Dr. Kelley's lab.

It is anticipated that within two years of the initiation of the study, Dr. Kelley would likely have enough evidence to publish a paper in a noted scientific journal. The results of this research would therefore establish a better understanding of the bioactivity of sweet cherries, help to establish a direction for future feeding trials or clinical research, while giving industry a study that it can promote immediately in its public relations efforts.

While the cost is not cheap, the SAB suggested that conducting the RMB study would be the easiest way to broaden our understanding of the product and would be an important first step in industry's research efforts. The plasma samples collected from their earlier feeding trials are invaluable and it is wise to gain as much information out of those samples as possible. **The SAB therefore recommended that industry pursue this project as their top priority.**

2. **Develop a Standardized Product to Aid Future Research** *Cost: Unknown*
SAB members agreed that one of the great challenges in doing research on fruit and vegetables in general is obtaining a shelf-stable product that can be standardized and available throughout the year. While doing whole fruit research is valuable, seasonal limitations and variations in fruit bioactivity from year to year, lot to lot, and variety to variety can challenge research results and restrict when and how studies can be conducted. Therefore, **the SAB recommends the cherry industry develop a freeze-dried product** that can be used for subsequent feeding trials.

It was mentioned that the table grape industry uses such a product in their research program. **The cherry industry should contact the California Table Grape Commission** to see if they would be willing to provide information about how they obtain their freeze-dried product, what steps they must undertake to ensure product consistency, and how they determine the relationship between shelf-life and bioactivity.

Due to the amount of variables that must be considered and the fact that fresh samples for freeze drying can only be collected during a short window during harvest, **industry should make it a priority to develop an action plan for this project before the 2009 harvest begins**.

3. **Chemical Analysis of the Fruit** *Cost: $20-30K + admin mark-up*
Part of the process of developing a standardized, freeze-dried product as noted above includes conducting a chemical analysis of the fresh fruit. After collecting a wide variety of samples from different locations, varieties, harvest timing, etc., the samples would undergo a chemical analysis using ORAC and FRAP to identify what compounds exist in the fruit and at what levels. Results would allow us to easily compare the chemical make-up of cherries with that of other fruits. For instance, POM says their product contains XYZ and therefore it is healthy. According to our chemical analysis, cherries have that too, so therefore cherries must also be healthy. According to the SAB, a chemical analysis of the fruit provides a basic understanding of the phytonutrients in cherries and how those nutrients might vary across a wide variety of samples. **If possible, this project should be conducted in concert with the efforts to develop a standardized, freeze dried product.**

4. **Dose Response Study** *Cost: $60-80K + admin mark-up*
Obtaining results from the chemical analysis is the first step in establishing a standardized dosage which is important for future research efforts. In particular, a dose response study gives the research community information about how much cherries a person needs to eat to achieve a certain reaction in the body. For instance, if only 30 cherries are needed to realize the result, then future feeding trials don't need to recommend 45 cherries. **A dose response study should take place only after industry completes the first three recommendations noted above.**

5. **Feeding Trials** *Cost: roughly $100K and up*
Assuming industry has done preliminary work to develop a standardized product and has established a clear dose response, feeding trials are the next step to learn more about how the body reacts to the different phytonutrients in sweet cherries. Although industry has previously conducted feeding trials without the benefit of having a more basic understanding of bioactivity and dose response, such information would likely strengthen future results. The SAB suggests that the media "loves" feeding studies, particularly if they are about a relevant topic. The SAB suggests that based on historical cherry studies, **industry should focus on arthritis, insulin resistance, and inflammation as primary areas of focus**. These are also relevant to consumers and could drive future demand if evidence linked cherry consumption to reduction or prevention of these diseases.

The SAB suggested that a researcher named Seerman NP might be a potential addition for the SAB. He has done a good deal of work on the anti-inflammatory effects of select anthocyanins in cell cultures and could help guide future feeding trials.

6. **Gene Array Sample** *Cost: $20-40K + admin mark-up*
   A gene array sample looks at how gene-specific nucleic acids react to various phytonutrients. In one test, 30,000 or more biomarkers can be analyzed to determine bioactivity. There was some debate among SAB members about when and if industry should pursue such a project. There was some consensus that **for any feeding trial, industry should plan to add on gene array analysis to get the broadest understanding of how the body reacted to cherry phytonutrients**.

7. **Epidemiological Study – Retro Data Analysis** *Cost: +$50K+ admin mark-up*
   According to Wikipedia, epidemiology is defined as

   > …the study of factors affecting the health and illness of populations, and serves as the foundation and logic of interventions made in the interest of public health and preventive medicine. It is considered a cornerstone methodology of public health research, and is highly regarded in evidence-based medicine for identifying risk factors for disease and determining optimal treatment approaches to clinical practice.[1]

   Instead of looking at the fruit and seeing what benefits it can bring to those that eat it, epidemiological studies look at a diseased population and understand how consumption of the fruit can reverse the progression. In other words, what does eating cherries do to those that suffer from arthritis?

   The SAB suggests industry can conduct a retroactive data analysis on existing food diary databases. With the right database and the right team to mine the data, industry could better understand how the bodies in a certain diseased population reacted to eating cherries. Apparently the data is there, we just need to get someone that can dig it up and knows how to interpret the results.

8. **Clinical Studies** *Cost: significant*
   Clinical studies are the gold standard of health research, but can be cost prohibitive. The length of the study and the amount of participants greatly impacts costs which could start at $500K and easily pass $1 million or more. Industry should consider the long-term impact that such an investment could have on future sales. However, industry should first conduct feeding trials and other pilot efforts to determine which areas could have the greatest chance of success.

   If industry is willing make such a large investment, it will want to almost guarantee results. If enough preliminary evidence is gathered, the National Institutes of Health (NIH) might decide to further explore the benefits of cherries. In which case, the federal government would foot the bill for the clinical work. **Industry should therefore revisit the idea of conducting a clinical study only after it has accomplished the preliminary steps previously recommended**.

The respective California and Northwest boards are now reviewing these recommendations and determining what funding to allocate to pursue one or more of these recommendations.

[1] <http://en.wikipedia.org/wiki/Epidemiology> Accessed on March 10, 2009

The CISP process has proven to be a positive experience for the U.S. cherry industry and will lead to a number of efficiencies between CCAB and WSFC. A comment made by one meeting participant summarizes the CISP experience for the U.S. sweet cherry industry: "I think we've come a long way. This is really great. I really like the honesty and it shows that we are all interested in working together." Clearly, the U.S. fresh sweet cherry industry is enthusiastic and committed to working together to increase global demand for U.S. cherries.