



# **BERRY HEALTH BENEFITS SYMPOSIUM**

The only conference focusing solely on berries & human health.

## **Symposium Pre-Proceedings**

2017 Berry Health Benefits Symposium  
March 28-30, 2017 | Pismo Beach, CA - USA

# The Berry Sessions

Tuesday, March 28th, 2017 - Grand Ballroom

8:30 - 9:15 AM - **Berryology 103 - Understand the Terminologies Used in Speaking About Berry Health** - Dr. Navindra Seeram



9:15 - 10:00 AM - **NHANES Report - Assessing Berry Fruit Consumption and Factors Associated with it in the United States** - Dr. Patricia Guenther



10:00 - 10:15 AM - **BREAK** - Lower Lobby



10:15 - 11:00 AM - **Berry Farmers Forum** - Berry farmers from different regions and crops discuss how research into berries and health supports the industry from a farmer's perspective

11:00 - 11:45 AM - **Marketing the Berry Health Message** - Collaborative presentation from the marketing teams of the major berry groups

12:00 - 1:00 PM - **LUNCH** - Waterfall Terrace - Sponsored by



## Scientific Presentations

Tuesday, March 28th, 2017 - Grand Ballroom

### Berry Special Topics, Food Technology & Chemistry

1:00 - 1:15 PM - **Current Research Review**. Chair overview by Dr. Navindra Seeram

1:15 - 1:40 PM - **The Missing Pink? Could Enterohepatic Circulation of Anthocyanins Help to Explain Their Health Benefits?** - Wilhelmina Kalt, PhD

1:40 - 2:05 PM - **Polyphenol-Enriched Berry Extracts Naturally Blunt Hyper-Reactive Proteins in Model Foods** - Mary Ann Lila, PhD

2:05 - 2:30 PM - **Insoluble Proanthocyanidin Interactions with Berry Cell Wall Components** - Christian Krueger, PhD

2:30 - 2:55 PM - **Cranberry Constituents Prevent Quiescence in the Uropathogenic Escherichia Coli Strain CFT073** - David Rowley, PhD

# Poster Session & Welcome Dinner

Tuesday, March 28th, 2017

**Poster Session - Zinfandel Room**

4:30 - 6:00 PM - Cocktail Hour Poster Session Presentations

Sponsored by



**Welcome Dinner - Main Lawn**

6:30 PM - Sponsored by Ocean Spray, Inc.



## Scientific Presentations

Wednesday, March 29th, 2017 - Grand Ballroom

### Berries, Heart & Healthy Aging

8:00 - 8:15 AM - **Current Research Review.** Chair overview by Dr. Britt Burton-Freeman

8:15 - 8:40 AM - **Cardioprotective Effects of Berries and the Probable Mechanism of Action** - Bahram Arjmandi, PhD, RD

8:40 - 9:05 AM - **Blueberries: Is it a “Berry” Good Idea for Cardiovascular Health?**  
- April Stull, PhD, RD

9:05 - 9:30 AM - **Evidence for Anti-Obesity and Beneficial Glucoregulatory Effects of Berries** - Janet Novotny, PhD

9:30 - 9:55 AM - **Berries and Bone Health: From In Vitro to Clinical Evidence** - Shanil Juma, PhD

10:00 - 10:15 AM - **BREAK** - Lower Lobby - Sponsored by



# Scientific Presentations

Wednesday, March 29th, 2017 - Grand Ballroom

## Berries and Metabolism

10:15 - 10:30 AM - **Current Research Review.** Chair Overview by Dr. Ron Prior

10:30 - 10:55 AM - **Role of Berries and Bioactives in the Modulation of Vascular Function: Evidence from In Vitro and In Vivo Studies** - Cristian Del Bo, PhD

10:55 - 11:20 AM - **Effects of Blueberries in a Preclinical Model of Post-Traumatic Stress Disorder** - Joseph Francis, BVSc, MVSc, PhD

11:20 - 11:45 AM - **Dietary Berries and Osteoarthritis (OA): Effects of Strawberries on Pain and Inflammation in Obese Adults with Radiographic Evidence of Knee OA** - Arpita Basu, PhD

11:45 - 12:10 AM - **Consumption of Red Raspberries, at Typical Levels of Intake Reduces Metabolic Syndrome Parameters in High-fat Fed Mice** - Ting Luo, PhD Candidate

12:15 PM - 1:30 PM - LUNCH - Waterfall Terrace - Sponsored by



## Berries and Brain Aging

1:30 - 1:45 PM - **Current Research Review.** Chair overview by Dr. Barbara Shukitt-Hale

1:45 - 2:10 PM - **Mitigating the Effects of High Fat Diet in the Brain with Berry Supplementation** - Amanda Carey, PhD

2:10 - 2:35 PM - **Effects of Flavonoid-Rich Blueberries on Cognitive Function in Healthy Younger and Older Adults** - Claire Williams, PhD

2:35 - 3:00 PM - **The Berry Flavonoid Fisetin is Protective in Multiple Animal Models of Age-Associated Neurological Disorders** - Pamela Maher, PhD

3:00 - 3:25 PM - **Berry Fruit Supplementation in Cognitive Aging: Advances in Human Berry Trials** - Robert Krikorian, PhD

# Poster Session & Keynote Dinner

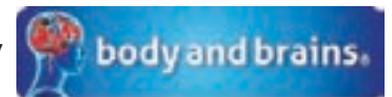
Wednesday, March 29th, 2017

**Poster Session - Zinfandel Room**

5:00 - 6:00 PM - **Poster Session Presentations** - sponsored by



5:30 - 6:30 PM - **Keynote Dinner Reception** - sponsored by



**Keynote Dinner - Grand Ballroom**

6:30 PM - Sponsored by Dole, Inc.



## Scientific Presentations

Thursday, March 30th, 2017 - Grand Ballroom

### Berries and Cancer

8:30 - 8:45 AM - **Current Research Review**. Chair overview by Dr. Ramesh Gupta

8:45 - 9:10 AM - **Berry Anthos for the Management of Various Cancers** - Farrukh Aqil, PhD

9:10 - 9:35 AM - **Mechanisms of Chemoprevention of Breast Cancer by Biofermented Blueberry Preparation: An Interface Between Nutrition and Cancer** - Chantal Matar, PhD, RD

9:35 - 10:00 AM - **Cranberry Fruits in Bladder Cancer Prevention** - Jeevan Prasain, PhD

10:00 - 10:15 AM - **BREAK** - Lower Lobby - Sponsored by



# Scientific Presentations

Thursday, March 30th, 2017 - Grand Ballroom

## Berries and Gut Health/Gut Microflora

10:15 - 10:30AM - **Current Research Review.** Chair Overview by Dr. Jess Reed

10:30 - 10:55 AM - **The Axis of Gut Bacteria-Metabolites-Their Receptors in Colon Carcinogenesis** - Li-Shu Wang, PhD

10:55 - 11:20 AM - **Raspberry Phytochemicals are Bioactive Following in Vivo Digestion** - Chris Gill, PhD

11:20 - 11:45 AM - **Ulcers, Stomach Cancer, Antibiotic Resistance and Cranberries: What's the Connection?** - Amy Howell, PhD

11:45 - 12:10 PM - **Cranberry Proanthocyanidins Reverse Microbial Dysbiosis and Inhibit Bile Acid Metabolism in Association with Esophageal Cancer Prevention** - Laura Kresty, PhD

12:10 PM - **BOXED LUNCH**

1:00 PM - **Tour buses depart from the front entrance of The Cliffs Resort**

1:30 PM - 9:00 PM - **California Berry Discovery Tour** - Travel by bus to Cal Poly & the berry growing regions of Central California and enjoy a final closing dinner.



# Keynote Address

“Riding the Health Wave and Connecting with 21st Century Berry Consumers”



## Professor David Hughes

Emeritus Professor of Food Marketing at  
Imperial College London

**Dr. David Hughes** is Emeritus Professor of Food Marketing at Imperial College London, and Visiting Professor at the Royal Agricultural University, U.K. He is a much sought-after speaker at international conferences and seminars on global food industry issues, particularly consumer and retail trends. David has lived and worked in Europe, North America, the Caribbean, Africa and South East Asia and has extensive experience as an international advisory board member with food companies and financial service organizations on three continents.

For 20 years, he was a Non-Executive Director of Berry Gardens Ltd – a U.K. farmer-owned berry fruit business (£250 million turnover in 2016). With his American business partner, David established, grew and sold a branded fresh produce business which served supermarkets in the USA. Around the globe, he works with food and beverage supply chain companies – including farm input, growers, manufacturers and ingredient companies, retailers and food service firms – to assist them in management training, strategy and Board level decision-making. David’s views are frequently sought by TV, radio and the printed press.

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## Tuesday, March 28th - The Berry Sessions



**Navindra Seeram, PhD**  
University of Rhode Island

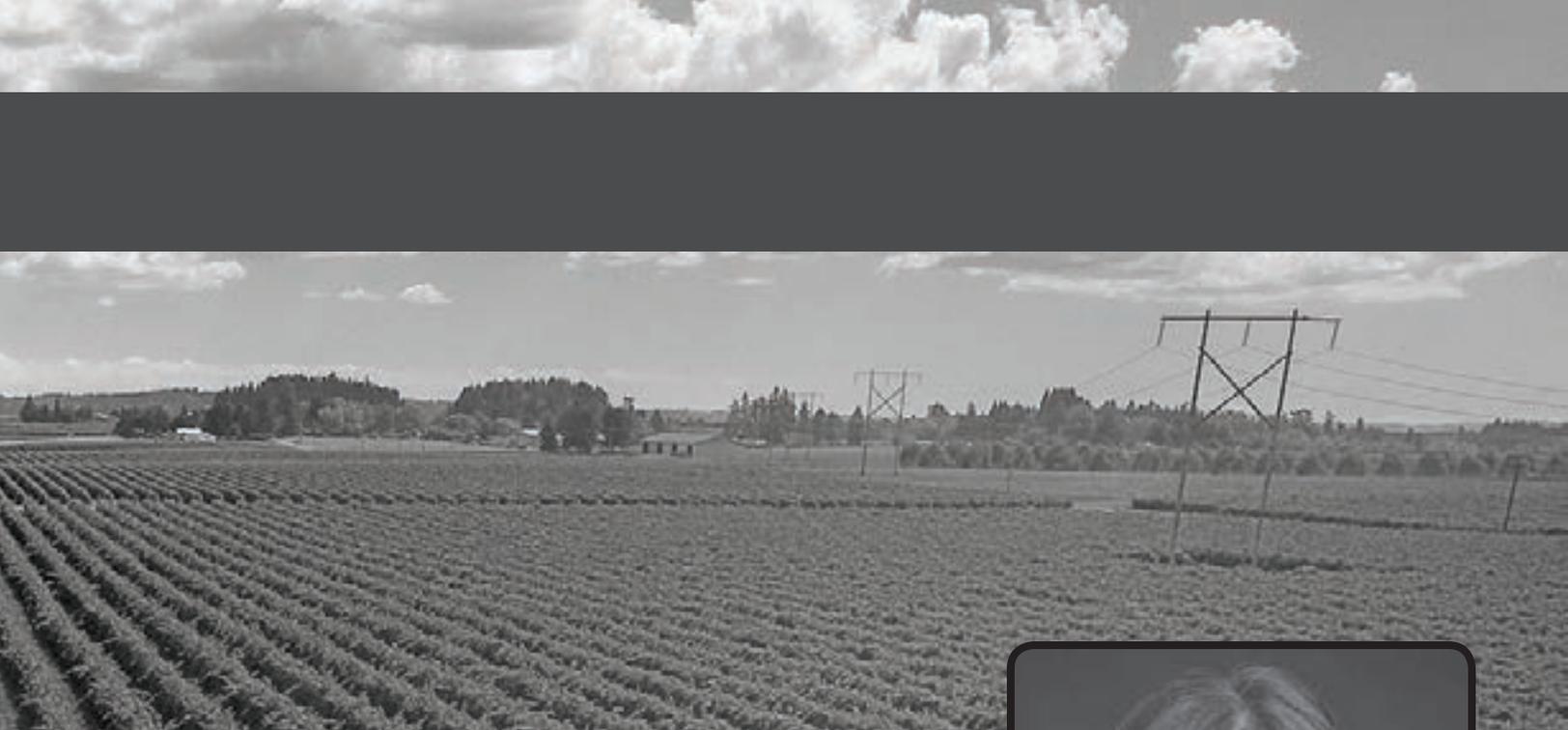
**Berryology 103 - Understand the Terminologies  
Used in Speaking about Berry Health**  
Co-Presenter: Britt Burton-Freeman

**Navindra P. Seeram, PhD**, is an Associate Professor in the Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, USA. Prior to this, he was the Assistant Director of the UCLA Center for Human Nutrition in the Department of Medicine, University of California at Los Angeles (UCLA), and an Adjunct Assistant Professor in the UCLA David Geffen School of Medicine.

His current research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants and their derived natural products for preventive and therapeutic effects against chronic human diseases.

Dr. Seeram has co-authored over 140 original peer-reviewed research articles, 8 review articles, 17 book chapters, and 6 international patents. He has co-edited 3 books and is the founding editor of the Clinical Pharmacognosy book series published by CRC Press/Taylor and Francis. He serves on the advisory board of the American Botanical Council and on the editorial advisory boards of the Journal of Agricultural and Food Chemistry, the Journal of Berry Research, and the International Journal of Applied Research in Natural Products. He was the recipient of the 2009 Young Scientist Award from the Division of Agricultural and Food Chemistry of the American Chemical Society and was elected as the 2017 Chair of that Division.

He is among the most highly cited scientists in Agricultural Sciences by Thomson Reuters (in 2014-2016 based on Web of Science indexed citations from 2002-2016) and is regularly quoted in the media and popular press about medicinal plant foods. Dr. Seeram did his doctoral and postdoctoral studies at the University of the West Indies (in Jamaica) and at Michigan State University (MI, USA), respectively.



**Patricia M. Guenther, PhD, RD**  
University of Utah

**NHANES Report – Assessing Berry Fruit  
Consumption and Factors Associated  
with it in the United States**



**Patricia M. Guenther, PhD, RD**, is a Research Professor in the Division of Nutrition at the University of Utah and Principle Consultant at Guenther Consulting. From 2003 to 2013, she was a nutritionist at the US Department of Agriculture’s Center for Nutrition Policy and Promotion, where conducted research related to dietary assessment and dietary guidelines and supported the development of the 2010 Dietary Guidelines for Americans.

Earlier Dr. Guenther was employed by the USDA Agricultural Research Service, where her work focused on developing improved methods for collecting and analyzing dietary survey data. Dr. Guenther received her B.S. and M.S. degrees in nutrition from Case Western Reserve University in Cleveland, Ohio, and her Ph.D. in Nutritional Sciences from the University of Maryland, College Park. She completed an internship in clinical dietetics at the University of Michigan Medical Center. Dr. Guenther is a registered dietitian, member of the Academy of Nutrition and Dietetics and the American Society for Nutrition, and serves on the editorial board of the Journal of the Academy of Nutrition and Dietetics.

#### **Summary of Project**

**Authors:** Patricia M. Guenther, Britt Burton-Freeman, David Stuart, Miyoung Oh, Helen Jensen

The goal of this paper is to expand the knowledge base about fruit consumption in the United States and explore the factors that may explain differences in fruit intake, especially berry intake, among sub-populations. This study will also determine how attitudes about diet and health influence fruit, and especially berry, consumption. The project takes advantage of the National Health and Nutrition Examination Survey (NHANES) database that includes a collection of 24-hour dietary recalls, known as “What We Eat in America,” and questions about knowledge, attitudes, and behaviors related to diet and health, known as the “Flexible Consumer Behavior Survey.” The 2007-2008, 2009-2010, 2011-2012 data collection cycles of NHANES are the most recent cycles available for analysis. The presentation will share results from the analysis and provide a forum for discussion of key learning points, actionable knowledge and areas we need focus future research.

## Berry Farmers Forum

Berry farmers from different regions and crops discuss how research into berries and health supports the industry from a farmer's perspective.



**Tom Avinelis**

Founder & President  
of Agri Care

Tom's expertise in grapes, citrus and blueberries has earned him a leadership role in the agriculture community. He has a Bachelor's Degree in Crop Science from California Polytechnic Institute, San Luis Obispo. A background rich with a variety of experiences ranging from pest control advisor to farm manager, research agronomist, crop consultant, sales manager, and director of various farming operations, has prepared Tom for both supportive and leadership roles in the Ag industry. He was an executive board member of California Citrus Mutual and also served as Vice President of the California Blueberry Association. Tom and his wife, Karen, founded AgriCare in 1990 which today specializes in agriculture asset management, farm management services and technical consulting. AgriCare currently manages over 14,500 acres in California and Oregon. In addition to his leading role in AgriCare, Tom was CEO of Homegrown Organic Farms from 2006 to 2013, an organic marketing company representing over 40 growers and more than 3,500 acres of organic produce. Tom remains President and Founder of AgriCare, Inc. Today Tom is a Principal at Agriculture Capital Management and works as a key member and developer for their permanent crop strategy to invest in permanent cropland and midstream assets to create a vertically integrated, sustainable farming enterprise that grows, packs and markets high-value produce.



**Ed Flanagan**

Owner / CEO of  
Jasper Wyman & Sons

Wyman's was founded in 1874 in Milbridge ME and is still privately owned by the Wyman family. Wyman's is the largest U.S. owned blueberry grower in the United States with farm and processing operations in Washington County Maine.

Wyman's grows wild (or lowbush) blueberries, as well as cranberries, and is one of the largest importers of other frozen fruits, principally from Chile and Mexico.

Ed Flanagan joined Wyman's in 1993 and has been President & CEO since 1995. Prior to that he worked for 12 years for H.P. Hood, Boston in dairy and citrus marketing, sales and general management responsibilities. He also worked in various sales and marketing roles for Ocean Spray Cranberries and for Waddington's.



**Bob Wilson**

Chairman of The  
Cranberry Institute

Bob has worked in the food industry for nearly 40 years and in 2005, he and his wife Evelyn opened the first of his current four companies representing the sales and marketing interests of independent cranberry growers. In 2008 Bob and two partners formed The Cranberry Network to manage sales for Habelman Bros. Cranberries, a fourth generation "fresh" cranberry grower and packer accounting for approximately 1/3 of the world's fresh cranberry supply. Bob is also a Managing Partner in Cranberry Partners, an industry leader in handling and marketing organic cranberries and a Managing Director of BNK Enterprises which manufactures and markets cranberry co-products. Bob is a Director of the Cranberry Institute and is currently serving as Board Chair. He and Evelyn reside in Wisconsin Rapids, WI.



**Adam Enfield**

Vice President of  
Enfield Farms

Adam owns and operates Enfield Farms Inc / Northwest Plant Company along with his Father Marv, and brother Andy. Enfield Farms Inc is a vertically integrated raspberry and blueberry farm in Northwest Washington State. It grows and packs approximately 700 acres of raspberries and 260 of blueberries.

Growing up on a farm, Adam has been working in the berry industry his whole life. He graduated from Seattle University with an undergraduate degree in Finance in 2002 and returned to the farm to continue his career. Well-versed in all operational areas of the farm, Adam's role has evolved to lead the Field Operations as a Vice President at the farm. He also serves as Co-General Manager for Northwest Plant Company. He currently serves as Research Chair, an elected position on the National Processed Raspberry Council that he has held since 2013.



**Greg France**

Owner / CEO of  
Mar Vista LLC

Born and raised in Calexico, California, Greg's lifelong dream was to always be a farmer. After graduating from Cal Poly, San Luis Obispo with a B.S. degree in Crop Science, Greg began his career in agriculture in Oxnard. Working in the vegetable and strawberry industry, his concentration with strawberries began in 1996. In 2004, Greg had the opportunity to farm 175 acres of strawberries for Coastal Berry. Today Greg farms approximately 1,000 acres of both conventional and organic strawberries for multiple shippers: Dole Berry, Wish Farms, Cal Fruit, Earthbound and Central West Produce, and in 2012, Greg was awarded the Santa Barbara County Farmer of the Year. He is a board director for the California Strawberry Commission, a grower representative for the Processing Strawberry Advisory Board, and a board director for the Boys & Girls Club of Santa Maria Valley. In his spare time, Greg enjoys biking, golfing and traveling.

## **Marketing the Berry Health Message**

Collaborative presentation from the marketing teams of the major berry groups

How does a health research project turn into a marketing initiative? Berry commodity groups have been investing into berry health research to help promote these crop for years. Hear from five commodity groups on how they take this research and promote it to different audiences. Closing the session, **Carrie Ann Arias**, VP of Marketing for Dole Fruit and Vegetables will give an industry brand perspective on the topic.



**Joanne Tehrani, MPH, RD, CdN**  
Manager, Nutrition Communications  
PadillaCRT



**McKenzie Hall Jones, RDN**  
Consultant for the National  
Processed Raspberry Council



**Terry Humfeld**  
Executive Director of  
The Cranberry Institute



**Kit Broihier, MS, RD, LD**  
Nutrition Advisor  
Wild Blueberry Assoc. of  
North America



**Jodi Reinman**  
Public Relations Manager  
California Strawberries



**CarrieAnn Arias**  
VP of Marketing  
Dole Fresh Vegetables & Berries

**Tuesday, March 28th - Scientific Presentations**



**Berry Special Topics,  
Food Technology  
and Chemistry**



**Navindra Seeram, PhD**  
University of Rhode Island

Session Chair

Current Research Review

**Navindra P. Seeram, PhD**, is an Associate Professor in the Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, USA. Prior to this, he was the Assistant Director of the UCLA Center for Human Nutrition in the Department of Medicine, University of California at Los Angeles (UCLA), and an Adjunct Assistant Professor in the UCLA David Geffen School of Medicine.

His current research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants and their derived natural products for preventive and therapeutic effects against chronic human diseases.

Dr. Seeram has co-authored over 140 original peer-reviewed research articles, 8 review articles, 17 book chapters, and 6 international patents. He has co-edited 3 books and is the founding editor of the Clinical Pharmacognosy book series published by CRC Press/Taylor and Francis. He serves on the advisory board of the American Botanical Council and on the editorial advisory boards of the Journal of Agricultural and Food Chemistry, the Journal of Berry Research, and the International Journal of Applied Research in Natural Products. He was the recipient of the 2009 Young Scientist Award from the Division of Agricultural and Food Chemistry of the American Chemical Society and was elected as the 2017 Chair of that Division.

He is among the most highly cited scientists in Agricultural Sciences by Thomson Reuters (in 2014-2016 based on Web of Science indexed citations from 2002-2016) and is regularly quoted in the media and popular press about medicinal plant foods. Dr. Seeram did his doctoral and postdoctoral studies at the University of the West Indies (in Jamaica) and at Michigan State University (MI, USA), respectively.



**Wilhelmina Kalt, PhD**  
Agriculture and Agri-Food Canada

**Wilhelmina Kalt** obtained her PhD from North Carolina State University and is currently employed with the Canadian federal agriculture department, Agriculture and Agri-Food Canada in the province of Nova Scotia.

Dr. Kalt's research on berry health benefits has focused on the anthocyanins of blueberry species. Her early work characterized the impact of horticulture and food factors on the antioxidant polyphenolics in berries. Dr. Kalt's work also included large-scale fractionation of blueberry fruit polyphenolics for bioactivity assessment in vitro and in vivo.

Dr. Kalt has conducted clinical and animal research on blueberries in the topic areas of visual function and anthocyanin bioavailability. Her recent findings on anthocyanin abundance and persistence in humans will support and inform clinical research using berry anthocyanins.

Willy works with industry groups and in particular the blueberry industries, to support the development of their health sector. She is very pleased to attend the 2017 BHBS.

## The Missing Pink? Could Enterohepatic Circulation of Anthocyanins Help to Explain Their Health Benefits?

New evidence of a large and complex pool of anthocyanin-derived flavonoid products may provide 'the missing pink' to explain in vivo berry health benefits. This evidence arises from a human feeding study (n=17) where more than 350 anthocyanin-derived products were detected in urine when blueberry juice was ingested daily for 28 days.

The pool of anthocyanin products was almost entirely de-glycosylated and was predominantly glucuronide conjugates. Excretion of food (i.e. parent) anthocyanins made up < 5% while about 50 anthocyanin metabolites accounted for 80%. Total 24-hour excretion was about 1% of the daily anthocyanin dose, which is approximately 20-fold greater than typical bioavailability estimates.

The significance of these anthocyanin-derived products owes to their C6-C3-C6 flavonoid structure that is associated with human health benefits. These products were highly persistent in the gastrointestinal tract (GIT) likely due to their enterohepatic circulation (EHC). Chronic exposure of the GIT and systemic circulation (via portal vein flow during EHC) to flavonoid intermediates supports growing evidence of berry anthocyanin health benefits.

Long-term persistence of anthocyanin products was demonstrated by (1) a 5-d anthocyanin-free run-in, (2) a 7-day washout and, in other studies (3) multiple week wash-outs in placebo-fed volunteers. Anthocyanin persistence in humans complicates the comparison of animal with clinical research evidence because animals will have had no previous dietary exposure to anthocyanins.

Large variation in urinary anthocyanin excretion among volunteers could be attributed almost entirely to human xenobiotic and physical factors and without involvement of GIT microbiota.

Results will be discussed in the context of clinical approaches to study anthocyanins in berry health research.

References:

**Keywords:** bioavailability, blueberry, flavonoid, LC-MS/MS

**References:**

1. Kalt et al., 2014, JAFC 62: 3926-3934
2. Kalt et al., 2017, JAFC, dx.doi.org/10.1021/jf500107j



**Mary Ann Lila, PhD**  
Plants for Human Health Institute  
North Carolina State University

**Mary Ann Lila** is Director of the Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus. She holds the David H. Murdock Distinguished Professorship, and is a Professor in the Department of Food, Bioprocessing, and Nutrition Sciences. Through ground-breaking, transdisciplinary discovery and outreach, her team of faculty at the Plants for Human Health Institute (PHHI) pioneers a dramatic shift in the way the American public views and uses food crops – not merely as a source of nutrients and flavorful calories, but as a powerful resource for components that protect and enhance human health. Integrated research in metabolomics, biochemistry, pharmacogenomics, molecular breeding, regenerative medicine, translational food science and nutrition and postharvest are aimed at development and promotion of mainstream fruit and vegetable produce with enhanced health benefits, and introduction of new or underappreciated crops and products from various sites throughout the globe, allowing consumers to make proactive, responsible dietary choices that benefit their own, and their families' health.

Dr. Lila is currently a co-Director of an ambitious public-private Plant Pathways Elucidation Project (P2EP) which synergizes the talents of academia and industry to unravel the complex genomics and metabolomics of functional food crops.

Recent projects include USDA-funded initiatives on polyphenol attenuation of food allergies, an NIH-sponsored project on berries and bone health, a Bill & Melinda Gates Foundation Grand Exploration Challenges project in Zambia, a NASA-sponsored project to develop stable functional protein-polyphenol colloidal particles to improve nutritive and sensory properties of portable food, an NIH NIDDKD project on functional food innovations; a major blueberry genome sequencing initiative using state-of-the-art NextGen sequencing capacity, which focuses on the genes relevant to health-protective properties in the fruits; and a USDA program on tribal resources and STEM education in American Indian/Alaska Native communities, the health protective properties of traditionally-used medicinal plants, and the threats imposed by climate change.

Lila was formerly Director (2006-2008) of ACES Global Connect (the international arm of the College of ACES, University of Illinois) and Associate Director of the nationally acclaimed Functional Foods for Health Program (1997-2000) at the University of Illinois. Dr. Lila has been honored with the Paul A. Funk Scholarship Recognition Award (the premier research award in the College of ACES, University of Illinois), the Spitze Professorial Career Excellence Award, the Faculty Award for Excellence in Research, the University Scholar Award, the Amoco Award for Excellence in Undergraduate Instruction, and the Lilly Endowment Teaching Fellowship. Dr. Lila has ongoing research projects in Australia, New Zealand, and various countries in Europe and Africa, and is Vice President of the Global Institute for BioExploration (GIBEX). In 1999, Dr. Lila won a Fulbright Senior Scholarship to conduct research and outreach in New Zealand, and returns to Australasia at least once/year.

# Polyphenol-Enriched Berry Extracts Naturally Blunt Hyper-Reactive Proteins in Model Foods

Today's consumers recognize and value the nutritive and health-protective benefits (such as satiety, muscle-building, disease prevention and weight management) afforded by foods containing lean proteins and phytochemically-rich berry fruits. However, modern consumers also increasingly must rely on portable pre-packaged convenience foods that complement their busy lifestyles. The challenge of delivering physiologically-relevant serving sizes of healthy ingredients in appetizing, convenient food products is exacerbated by the natural reactivity of protein molecules. Reactive proteins can provoke human receptors (triggering food allergies) or form inter-molecular networks with other food ingredients (destabilizing food structures and causing aggregation or hardening in high protein bars).

By capitalizing on a natural chemical affinity for berry phytoactive polyphenols to bind to healthy edible proteins, highly-functional protein-polyphenol colloidal aggregate particles (with deliberately blunted protein reactivity) were designed and evaluated. Berry or berry pomace extracts were complexed with a series of edible proteins (whey, rice, soy, peanut, egg) to form chimeric micrometer-scale three-dimensional aggregates, bound both by covalent and hydrophobic (non-covalent) forces depending on formulation process. The highly colored, concentrated colloidal suspensions were comprised of a range of particle sizes and anthocyanin (10-70 mg g<sup>-1</sup>) total polyphenol (15-100 mg g<sup>-1</sup>) or proanthocyanidin (20-150 mg g<sup>-1</sup>) concentrations depending on the protein source, complexing process conditions and concentrations of starting materials.

**Modulation of immunoreactivity.** This novel, food-grade, green chemistry approach leverages two different strategies for alleviation of food allergies. First, polyphenols alone have the ability to inhibit chemical mediator release in allergy. Second, the colloidal aggregates can interfere with allergenicity by masking and/or changing the conformation of the allergenic protein epitopes.<sup>1,2</sup> When food proteins were complexed with polyphenols from blueberry, cranberry, aronia, or blackcurrant, immunoblotting with plasma from allergic patients demonstrated significantly decreased IgE binding, indicative of reduced food allergenicity.<sup>1</sup> Protein-polyphenol particles triggered significantly less basophil degranulation (ex vitro) and mast cell degranulation (in vivo) as compared unmodified peanut flour, with both treatments normalized to the same amount of protein challenge. Peptides from peanut-protein-cranberry polyphenol colloidal aggregates were substantially less immunoreactive and more quickly hydrolyzed during simulated gastric digestion than those from uncomplexed peanut protein.

**Food Functionality.** A compelling challenge for the food manufacturing industry is to reconcile consumer demand for healthier foods (e.g. proteins and plant-derived polyphenols) with the simultaneous demand for convenience and product quality. Whereas aggregation between protein molecules can lead to destabilization, phase separation, or cross linking and hardening of high protein food products, the protein-polyphenol particles did not participate in these aggregation events, even over a long storage time. Whey protein-cranberry polyphenol particles ranging from 1-100  $\mu$ m diameter (1-2 mg polyphenol per g and a molar ratio of 9-50 proteins per polyphenol) dramatically improved foam stability (decreased drainage rate) as compared to foams prepared with whey protein alone.<sup>3</sup> When only a fraction (20-30%) of the densely-packed protein molecules in a bar were delivered in the form of protein-polyphenol particles, the typical hardening and cross-linking was significantly inhibited. When used as a food ingredient, these food-grade protein-polyphenol particles deliver both health functionality, and structural functionality to stable food formulations.

**Key Words:** protein-polyphenol colloidal aggregate, food allergy, protein epitope, health functionality, food structural functionality

## References:

1. Plundrich, Nathalie, Mike Kulis, Brittany L. White, Mary H. Grace, Rishu Guo, A. Wesley Burks, Jack P. Davis and Mary Ann Lila. 2014. Novel strategy to create hypoallergenic peanut protein-polyphenol edible matrices for oral immunotherapy. *Journal of Agricultural and Food Chemistry* 62:7010-7021. <http://dx.doi.org/10.1021/jf405773b>
2. Plundrich, Nathalie, Brittany White, Lisa L. Dean, Jack P. Davis, E. Allen Foegeding, and Mary Ann Lila. 2015. Stability and immunogenicity of hypoallergenic peanut protein-polyphenol complexes during in vitro digestion. *Food & Function* 6: 2145-2154. DOI: 10.1039/C5FO00162E
3. Schneider, Margaret, Debora Esposito, Mary Ann Lila and E. Allen Foegeding. 2016. Formation of whey protein-polyphenol meso-structures as a natural means of creating functional particles. *Food & Function* 7:1229-1318. DOI: 10.1039/C5FO01499A PMID 26857696



**Dr. Christian Krueger, PhD**  
Complete Phytochemical Solutions, LLC

**Mr. Krueger** is the Chief Executive Officer and Co-Founder of Complete Phytochemical Solutions, LLC, a consulting and analytic service company that provides intellectual and technical expertise in phytochemistry that enables their clients to develop, manufacture and market high quality and efficacious botanical and food products for human and animal nutrition.

Mr. Krueger is also Principal Investigator and Director of Operations for the Reed Research Group's basic and translational research program at the University of Wisconsin-Madison. The Reed Research Group embodies three core competencies: Phytochemistry, Cardiovascular Disease and Mucosal Immunity.

Mr. Krueger pioneered the development of MALDI-TOF mass spectrometry techniques for characterization of the structural heterogeneity of oligomeric polyphenols in fruits, beverages and nutritional supplements. Analytic tools such as this are currently used to support authenticity, standardization and efficacy evaluation of natural products.

# Insoluble Proanthocyanidin Interactions with Berry Cell Wall Components

There is an ever-increasing diversity of berry products entering the food and dietary supplement markets. Manufacturers, health researchers and consumers rely on the development of new analytic methods to support the authentication, standardization and health benefits of these berry products. The presentation entitled 'Insoluble Proanthocyanidin Interactions with Berry Cell Wall Components' will focus on the discovery of a new class of compounds in cranberry fruit, insoluble proanthocyanidins (PAC). New analytic methods for the quantification of the insoluble PAC will be presented and future research opportunities on the health benefits will be discussed.

Proanthocyanidins found in cranberry fruits and commercial products are partitioned into two categories, soluble PAC (extractable) and insoluble PAC (non-extractable). The history of how the cranberry sample was handled and processed, dictates the relative proportioning of these two classes of PAC. Products such as cranberry juice and spray dried juice powders contain almost exclusively soluble PAC. Fresh and frozen cranberry fruit, sweetened dried cranberries and juice manufacturing co-products such as; press cake, seeds, and skins contain both soluble PAC and insoluble PAC. There are also numerous dietary supplement products that are formulated to include both soluble and insoluble PAC.

The cranberry industry has recently adopted the 4-(Dimethylamino) cinnamaldehyde (DMAC) analysis for quantification (how much is there) of soluble PAC.<sup>1,2,3</sup> Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is recognized as a 'fit-for-purpose' analysis for the authentication (what does it look like) of specific soluble PAC structural features, such as A-type interflavan bonds.<sup>4,5,6</sup> Insoluble PAC cannot be analyzed by these same methods due to their non-extractable nature, and thus represent an underappreciated and uncharacterized proanthocyanidin component of many cranberry products

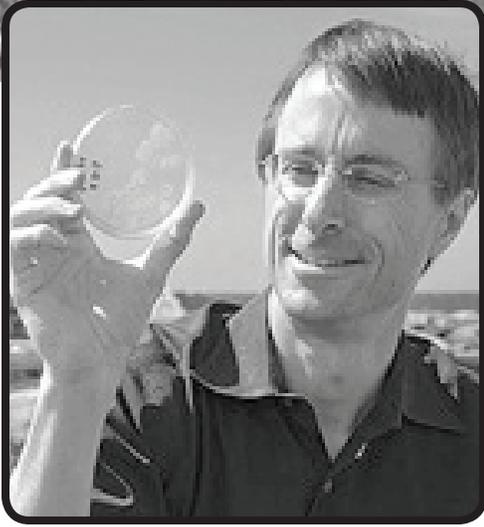
Despite their relatively recent discovery in cranberry products, insoluble PAC were described to exist in other plants (i.e. East African browse species) many decades ago.<sup>8,9</sup> Insoluble PAC are bound to plant cell wall components such as fiber or protein, and as a result are not readily extracted during juicing processes or solvent extraction. Insoluble PAC have a higher affinity to the solid fruit cell wall materials than they do to the liquid in which they are being extracted. Thus, insoluble PAC are an important but underappreciated class of compounds and limited, but steadily growing knowledge, exists on their health benefits.

The results of recent work indicate that the butanol-HCl (BuOH-HCl) assay, when used with an appropriate cranberry proanthocyanidin standard (c-PAC), can quantify insoluble PAC. The adoption of the BuOH-HCl method for insoluble PAC analysis will allow for a more comprehensive characterization of cranberry products. The ability to quantify soluble and insoluble PAC will enable manufactures to better control of the effects that harvesting, storage and processing have on PAC composition. Furthermore, the quantification of insoluble PAC associated with the fiber fractions of cranberry products (and other PAC containing berries) will enable researchers to develop and test new hypothesis on the bioactivity of this class of PAC in relation to health and nutrition.

**Key Words:** Cranberry, Insoluble Proanthocyanidin, polyphenol-fingerprinting, butanol-HCL

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**Dr. David Rowley**  
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Professor of Biomedical and Pharmaceutical Sciences, Alex & Ani Positive Impact Laboratory,  
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**David Rowley** obtained his PhD at the University of California - San Diego where he carried out research on antiviral secondary metabolites produced by marine microorganisms and plants. Since 2001, he and his students at the University of Rhode Island have investigated natural products from both the marine and terrestrial environments as new tools to combat infectious diseases. David's current research interests include molecules that can prevent and disrupt microbial biofilms, compounds that interfere with cell-cell communication in bacteria, and methods to reverse antibiotic resistance. In collaboration with Ocean Spray Cranberries, David is currently investigating molecular mechanisms by which cranberry constituents prevent urinary tract infections.

# Cranberry Constituents Prevent Quiescence in the Uropathogenic Escherichia Coli Strain CFT073

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Urinary tract infections (UTI) commonly occur in the kidney and bladder. UTI patients often experience frequent recurrence and increasing susceptibility to drug resistant uropathogens (1). Over 80% of UTIs are associated with uropathogenic Escherichia coli (UPEC), which can invade urothelial cells and create reservoirs of bacteria that are shielded from antibiotics and the host immune system (2-4). Once inside bladder cells, UPEC appear to enter a non-growing, quiescent intracellular state. It is thought that these 'quiescent intracellular reservoirs' are a major cause of recurrent UTIs (5,6). Using a newly developed assay for the study of E. coli quiescence (7), we discovered that specific, non-phenolic constituents of cranberry (Vaccinium macrocarpon) prevent quiescence in a the UPEC strain CFT073. These results encourage further investigation of certain cranberry constituents for prevention of recurrent urinary tract infections.

**Key Words:** Cranberry, Urinary Tract Infection, E. coli, quiescence

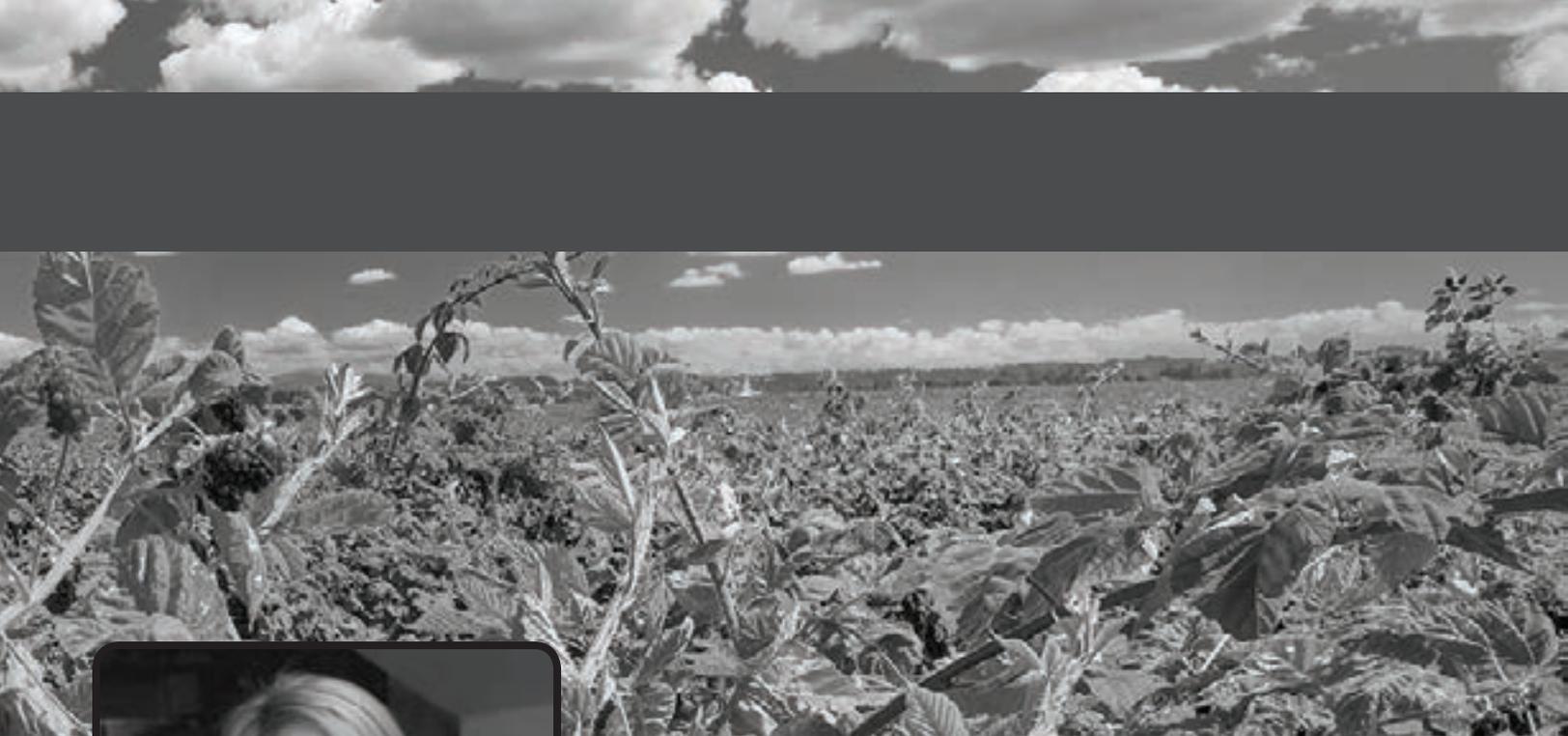
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**Wednesday, March 29th - Scientific Presentations**



## **Berries, Heart and Healthy Aging**



**Dr. Britt Burton-Freeman**  
Illinois Institute of Technology

Session Chair

Current Research Review

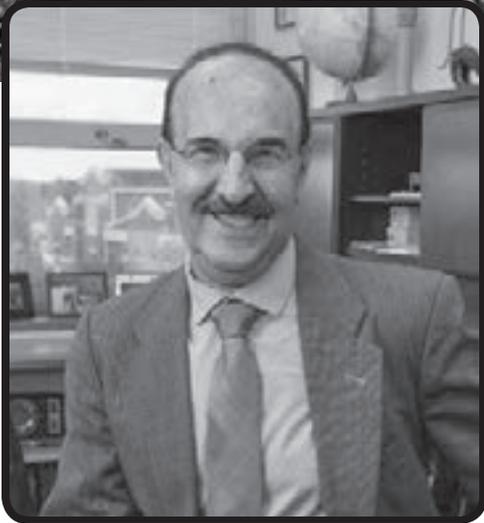
**Britt Burton-Freeman, Ph.D.**, is the Director of the Institute for Food Safety and Health's (IFSH) Center for Nutrition Research and Associate Professor in Food Science and Nutrition and Biomedical Engineering at the Illinois Institute of Technology (IIT). She also holds a research nutritionist appointment in the department of Nutrition at UC Davis and is affiliated with the Institute for Translational Medicine at the University of Chicago.

Dr. Burton-Freeman's current research interests are in mitigating disease processes through dietary approaches focused on bioactive components of foods. Specific disease targets are cardiovascular, metabolic syndrome and obesity. Current work focuses on physiological effects and mechanistic underpinnings of polyphenols and novel carbohydrates, including their pharmaco-kinetic and -dynamic relationships in human biology to impact health status. The influence of food matrix, processing, host/microbiome characteristics and interactions are also being addressed.

As the Director for the Center for Nutrition Research at IIT/IFSH in conjunction with the National Center for Food Safety and Technology, she leads a nutrition and health initiative with food industry partners and government collaborators to provide critical science that supports policy, dietary recommendations and comprehensive innovative solutions linking nutrition and food safety to improve the health and quality of life of Americans. Recent work has focused on fiber definitions for labeling and perceptions/responses to key terms associated with health in low income populations.

Dr. Burton-Freeman is actively involved in multiple professional societies dedicated to health and disease abatement including the American Society for Nutrition, the Obesity Society, the American Chemical Society and the Institute of Food Technologist. Dr. Freeman publishes in various top Journals and is co Editor-in-Chief of Nutrition and Healthy Aging.

Dr. Burton-Freeman holds a BS in Dietetics from the California State University, Chico, a MS and PhD in Nutritional Biology from the University of California, Davis and completed a postdoctoral fellowship in the Department of Internal Medicine at University of California, Davis. Dr. Burton-Freeman has held professional appointments in academia and the biotechnology industry leading research programs and teams to deliver on basic and clinical science objectives.



## **Bahram H. Arjmandi, PhD, RD** Florida State University

**Bahram H. Arjmandi, PhD, RD** is currently the Margaret A. Sitton Named Professor at Florida State University (FSU) and is the founder and Director of the Center for Advancing Exercise and Nutrition Research on Aging (CAENRA) at FSU. He has also served in numerous capacities at FSU, including being a member of the FSU Biomedical Advisory Committee, the Council on Diversity and Inclusion, and as the chair of the Department of Nutrition, Food and Exercise Sciences for eight years. Dr. Arjmandi is a Registered Dietitian who received his Ph.D. from the Department of Human Nutrition at Kansas State University where he studied the effect of soluble fiber on sterol synthesis and later completed his postdoctoral work in the area of estrogen and bone physiology at the University of Texas Health Science Center. His current research emphasis is women's health including cardiovascular health, osteoporosis, and osteoarthritis. In recognition of his accomplishments in women's health, he received the Abbott Nutrition Award in Women's Health in 2013. He was one of the first investigators to provide evidence for estrogen receptors in the gut to aid in calcium transport and to demonstrate the efficacy of dried plum in protecting bone in both animal models of osteoporosis and postmenopausal women. He has also conducted clinical studies examining the beneficial effects of berries, including blueberries, strawberries, raspberries, and blackberries on cardiovascular health. He has received grants from USDA, NIH, NASA and state agencies to support his research and has also served as a panel member for NIH and panel member and panel manager for USDA/NRI. Dr. Arjmandi was one of the twelve invited US forum delegates to the Traditional Indian Systems of Medicine Symposium sponsored by the NIH and the Indian government. He has published more than 125 peer-reviewed journal articles and has received numerous recognitions for his scholarly research and graduate student advisement including the Margaret Scruggs Award for Meritorious Research, the Regents Distinguished Research Award at Oklahoma State University (OSU), and Distinguished Research Award at Kansas State University.

In addition to his research endeavors, Dr. Arjmandi was awarded the Outstanding Mentor Award three times at OSU and was recognized as an Outstanding Alumni from the College of Human Ecology at Kansas State University, and was the recipient of The Dr. Masoro Outstanding Alumnus Award from the University of Texas Health Science Center in 2012.

Dr. Arjmandi is serving as Editor-in-Chief for the Journal of Food & Nutrition Disorders and as editorial board member of several other journals including the Journal of Diabetes Mellitus and Preventative Nutrition and Food Science. He is also a member of several worldwide organizations including the International Bone and Mineral Society and the North American Menopause Society.

## Cardioprotective Effects of Berries and the Probable Mechanism of Action

Cardiovascular disease (CVD) is a major public health concern and the leading cause of death in the United States (US). The incidence of CVD increases significantly with age in both men and women, while the onset of menopause places women at a higher risk of developing CVD and mortality. Aging and sex-hormone-deficiency are characterized by increased levels of reactive oxygen species (ROS) and pro-inflammatory molecules. Although a number of medications are available to treat CVD, lifestyle interventions including dietary modifications are the primary recommended approaches for prevention and treatment of CVD. Animal and human studies have shown that consumption of certain foods, in large part due to their bioactive compound content, can reduce the risk of CVD. Among fruits, berries are an excellent source of polyphenols which have strong antioxidant and anti-inflammatory properties and have also been shown to be cardio-protective. For instance, we demonstrated that daily consumption of freeze-dried blueberry powder (22 g) for eight weeks decreased blood pressure and arterial stiffness and increased circulating nitric oxide metabolites in postmenopausal women with pre- and stage 1-hypertension (1). In addition, we recently examined the antihypertensive and vascular-protective properties of strawberries in the same population. Systolic blood pressure and arterial stiffness were decreased in the group consuming freeze-dried strawberry powder (25 g) compared to baseline but no differences between groups were detected (2). No changes were noted in the placebo-control or 50 g freeze-dried strawberry powder groups. Additionally, we have investigated the mechanism(s) through which berries exert their cardio-protective effects. We recently reported that blackberry, raspberry, and black raspberry polyphenol extracts attenuate angiotensin II-induced increases in ROS and senescence in vascular smooth muscle cells (VSMCs) (3). Blackberry polyphenol extract attenuated the up-regulation of NOX-1 expression while increasing the expression of superoxide dismutase (SOD)1. On the other hand, raspberry and black raspberry polyphenol extracts up-regulated the expression of SOD1 and SOD2 as well as glutathione peroxidase 1. Altogether, our findings suggest that berries may improve blood pressure and arterial stiffness by counteracting ROS levels. Thus, the purpose of this presentation is to provide an overview of the studies that we conducted to investigate the effect of berries on lipid profiles, atherogenic risk ratios, proinflammatory markers and oxidative stress markers.

**Key Words:** berries, cardiovascular disease, polyphenols, blood pressure, aging

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**Dr. April J. Stull, PhD, RD**  
University of Maryland

**Dr. Stull** is an Associate Professor of Nutrition in the Department of Human Ecology at the University of Maryland Eastern Shore. Her research focuses on botanicals and their impact on risk factors associated with metabolic syndrome. Specifically, Dr. Stull's lab found that supplementation with blueberries for 6 weeks improved insulin sensitivity and endothelial function in adult obese men and women that had metabolic syndrome. Dr. Stull received federal (National Institutes of Health, National Center for Complementary and Integrative Health) and nonprofit (US Highbush Blueberry Council) funding to support her blueberry research. She has published numerous book chapters and manuscripts in peer-reviewed scientific journals related to the health benefits of blueberries. Additionally, she has presented her research findings at many international and national conferences. Dr. Stull is very involved in various professional organizations, such as the American Society for Nutrition (Chair 2013-2014, Young Professional Interest Group; Vice Chair 2015-present, Minority and Diversity Affairs Committee), American Diabetes Association, and NIDDK Network of Minority Health Research Investigators.

Dr. Stull is also a Registered Dietitian and attained her Ph.D. in Nutrition Science from Purdue University. Her graduate training was followed by a National Institutes of Health T32 postdoctoral fellowship in diabetes, nutrition, and botanicals at Pennington Biomedical Research Center (Botanical Dietary Supplements Research Center, Baton Rouge, LA).

During her fellowship, she received the MARC (Maximizing Access to Research Careers) Postdoctoral Professional Development and Enrichment Award. Recently, she was honored as a Diamond of the Department of Nutrition Science at Purdue University. This award recognized her contributions to advancing the field of Nutrition. In her spare time, Dr. Stull enjoys spending time with family and friends, traveling, and scrapbooking.

## Blueberries: Is it a “Berry” Good Idea for Cardiovascular Health?

The risk factors for cardiovascular disease (CVD) encompass insulin resistance, central obesity, dyslipidemia and hypertension, which are also features of metabolic syndrome (1). These risk factors contribute to vascular abnormalities, such as endothelial dysfunction, which represents a very early step in the process of atherosclerosis and is also associated with increased adverse CVD outcomes (2). Endothelial dysfunction can be improved, and some studies indicate that the risk of developing endothelial dysfunction increases with the number of risk factors present in an individual (3,4).

Blueberry consumption has been shown to have various health benefits in humans. We have previously shown that the consumption of anthocyanin-rich blueberries improved insulin resistance (5), a known CVD risk factor. To further investigate the health benefits of blueberries in humans, we investigated the role of blueberry consumption on modifying blood pressure and endothelial function in subjects with metabolic syndrome (6). A double-blind and placebo-controlled study was conducted in 44 adults (blueberry, n = 23; and placebo, n = 21). They were randomized to receive a blueberry or placebo smoothie twice daily for six weeks. Endothelial function was assessed using the non-invasive EndoPAT™ 2000 device (Itamar Medical Ltd.) pre- and post-intervention. Furthermore, 24-hour ambulatory blood pressure was monitored over seven days using an automatic blood pressure monitoring device (Tiba Medical, Inc.) pre- and post-intervention. Six weeks after consuming the blueberry or placebo smoothie, the blood pressure did not differ between the groups. However, the mean change in resting endothelial function, expressed as reactive hyperemia index (RHI), was improved significantly more in the group consuming the blueberries versus the placebo group ( $p < 0.05$ ). Even after adjusting for confounding factors, i.e., the percent body fat and gender, the blueberry group still had a greater improvement in endothelial function when compared to their counterpart ( $p < 0.05$ ). In conclusion, daily dietary consumption of blueberries for six weeks did not improve blood pressure, but improved (i.e., increased) endothelial function in a population at high risk for developing CVD.

**Keywords:** blueberries; endothelial function; endothelial dysfunction; prediabetes; hypertension; cardiovascular risk factors

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**Dr. Janet A. Novotny**  
U.S. Department of Agriculture's  
Human Nutrition Research Center

**Dr. Janet A. Novotny** is a Research Physiologist with the U.S. Department of Agriculture's Human Nutrition Research Center in Beltsville, Maryland. Holding degrees in mathematics, nutrition, and biophysics, Novotny combines her areas of expertise to conduct studies on bioavailability, metabolism, and health benefits of dietary components.

Novotny's research involves several facets of the relationship between diet and health. One aspect of Novotny's work is the bioavailability, pathways of metabolism, and rates of elimination of phytonutrients and micronutrients. Novotny conducts human intervention studies which combine technologies of stable isotopes, mass spectrometry, and mathematical modeling to assess nutrient absorption and pathways of metabolism. Novotny has published on the pharmacokinetics of anthocyanins, carotenoids, vitamin A, vitamin K, vitamin E, and molybdenum. Novotny also conducts clinical studies to assess mechanisms by which dietary bioactive components, especially polyphenols, reduce risk of chronic disease, including cancer, diabetes, and cardiovascular disease.

Novotny earned a B.S. in mathematics, an M.S. in Nutritional Sciences, and a Ph.D. in Biophysics from the University of Illinois, and is an Adjunct Professor in the Department of Nutrition at the University of Maryland. She is active in the American Society for Nutrition, has served as an Associate Editor for Crop Science, is the Government Liaison for the International Life Sciences Institute Committee on Bioactives, and has edited two books on Mathematical Modeling.

## Evidence for Anti-Obesity and Beneficial Glucoregulatory Effects of Berries

While there have been some mixed results with respect to the potential role of berries in combatting obesity, there are still a notable number of animal studies suggesting that foods rich in anthocyanins may protect against weight gain and/or body fatness [1-14]. These studies have included a number of different berries, including blueberry [6, 10], strawberry [4], blackberry [14], mulberry [11], as well as other foods rich in anthocyanins, such as cherry [2, 5, 13], purple corn [1], blood orange [7], and black soybean [3, 9]. Generally, these studies have been performed in animal models consuming a high fat diet. In most cases, decreases in food intake do not appear to account for the difference in weight gain and adiposity when anthocyanin sources are included in the diet [1-3, 5, 11, 13, 14].

One experimental approach for translating these findings to humans is indirect calorimetry. Indirect calorimetry uses the respiratory quotient, which is the ratio of the carbon dioxide produced to the oxygen consumed, to determine energy substrate utilization. At the USDA Beltsville Human Nutrition Research Center, indirect calorimetry was used to translate these positive findings for berries from animal studies to humans. For the study, healthy, overweight men spent 24 hours in a room-sized calorimeter. Prior to the calorimeter stay, the volunteers consumed a fully controlled, high fat diet with or without blackberries for one week. Data collected from the calorimeter included volumes of carbon dioxide produced and oxygen consumed, providing information about which of the metabolic fuels the body was using. A meal-based glucose tolerance test was also administered. The study revealed that blackberry consumption resulted in increased fat oxidation over the 24 hour stay in the calorimeter, as well as during several isolated time increments during the 24 hour test period. Volunteers also appeared to exhibit improved insulin sensitivity after consumption of blackberries.

Overall, there is some inconsistency among studies as to the role of berries in reducing body weight and adiposity. Reasons for the inconsistency may include differences in study design, berry or anthocyanin source, experimental model, background diet, or other study details. However, the body of evidence suggesting that berries may help protect against body weight gain and adiposity continues to grow, warranting further studies to determine under what conditions berries can be most effective for improving health.

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**Shanil Juma, PhD**  
Texas Woman's University

**Shanil Juma's** main research interest is investigating the etiology of age-related conditions, osteoarthritis and osteoporosis, as a basis for the development of effective nutritional strategies for the prevention and management of these disorders. These research projects employ analytical, biochemical, and molecular techniques using cell culture and animal models, as well as small-scale clinical trials. The focus of these investigations is to elucidate the anti-inflammatory, bone, and joint protective properties of naturally occurring bioactive compounds present in whole foods (functional foods, e.g. blueberries, raspberries, grape, tart cherries etc.).

Dr. Juma's secondary research focus is on obesity and obesity-related metabolic conditions (diabetes, cardiovascular, etc.). Current studies are focused on food components such as resistant starch, spices (curcumin, cinnamon, etc), and berry polyphenols (blueberry, raspberry, strawberry, etc.) on weight management, glucose homeostasis, and gut health.

## Berries and Bone Health: From In Vitro to Clinical Evidence

Osteoporosis is a disease characterized by an imbalance in skeletal homeostasis in which bone resorption exceeds bone formation (1). The resulting loss of bone mass compromises bone strength leading to an increased risk for fractures (2). Osteoporosis is most common among the older population in Western societies, especially in postmenopausal women (1). It is estimated that this disease currently impacts more than 200 million individuals worldwide and is responsible for over 8.9 million fractures each year. Nearly half of all these fractures are reported to occur in Europe and the Americas (3). According to the National Osteoporosis Foundation, greater than 9.9 million Americans are afflicted by the disease and an additional 43 million are at an increased risk for osteoporosis due to low bone density. These numbers are anticipated to increase as the aging population continues to grow. The occurrence of fractures places substantial economic and social burden on the individual who experiences them (4). Approximately two million fractures annually are attributed to osteoporosis in the United States, which represents nearly \$20 billion in medical costs each year (5,6). These costs are expected to rise to \$25.3 billion by 2025 (4). Therefore, the investigation of the preventive measures that could be useful in protecting against this debilitating disease is crucial.

Inflammation is implicated in the development of chronic diseases, including osteoporosis (7). Chronic inflammation can increase oxidative stress within the body due to overproduction of free radicals and cytokines (8). Free radicals promote bone loss by increasing osteoclast activity and enhancing the production of inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1). TNF- $\alpha$  and IL-1 have been associated with osteoclast differentiation and activity (9). These cytokines have been shown to influence osteoclastogenesis directly through signaling pathways and indirectly by promoting the production of other inflammatory markers (10,11). Thus, chronic inflammation enhances bone loss, which can contribute to the development of osteoporosis.

Epidemiological studies have implicated that consumption of a diet rich in fruits and vegetables may be beneficial to skeletal health. Plant-based foods are known to be good sources of bioactive polyphenolic compounds (7). Recent in vitro studies have investigated the potential mechanisms by which these compounds may positively affect bone metabolism (12-17). Evidence suggests that polyphenols can inhibit osteoclast differentiation and activity through their action as antioxidants. Antioxidants scavenge for free radicals and can subsequently downregulate the production of inflammatory molecules. Thus, polyphenols may contribute to bone health by counteracting the inflammatory process (7).

Blueberries and raspberries contain a rich mixture of polyphenolic compounds such as anthocyanins, proanthocyanidins, and chlorogenic acid. In-vivo and in-vitro studies have demonstrated that these polyphenols inhibit the inflammatory response (12-19) and suggest that these compounds may be effective in protecting bone as observed in limited animal studies (18-19). This presentation will focus on the in vitro findings associated with blueberry and red raspberry polyphenols in murine bone cells and the outcomes of two short term clinical studies investigating whole blueberry and red raspberry consumption on bone health in postmenopausal women.

**Keywords:** Osteoporosis, Fracture, Blueberry, Raspberry, Polyphenols, Inflammation

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**Wednesday, March 29th - Scientific Presentations**

## **Berries and Metabolism**



**Dr. Ron Prior**  
University of Arkansas  
Session Chair  
Current Research Review

**Dr. Prior** received his Ph.D. in Nutrition with minors in biochemistry and physiology from Cornell University. His graduate training was followed by two years of post-doctoral training in Comparative Gastroenterology through the College of Veterinary Medicine at Cornell University. Dr. Prior was with the Agricultural Research Service of the USDA for 35 years. Following 13 years at the USDA Human Nutrition Research Center on Aging at Tufts.

Dr. Prior moved in 2000 to the USDA Arkansas Children's Nutrition Center in Little Rock, AR where he provided leadership for their phytochemical and health research program. In May of 2010 Dr. Prior retired from the USDA, but he continues to serve as adjunct professor in the Dept of Food Science at the Univ. of Arkansas, Fayetteville and to consult with organizations on matters related to phytochemicals and nutrition.

Dr. Prior has published more than 220 articles in peer reviewed scientific journals. Dr. Prior received the Alex Wetherbee Award from the North American Blueberry Council for his contributions to the blueberry industry. In 2006, was ranked as the top-cited author in agricultural sciences by Science Watch.



**Cristian Del Bò, PhD**  
University of Milan

**Cristian Del Bò, PhD** works at the Department of Food, Environmental and Nutritional Sciences, at the University of Milan, Italy. He graduated in Food Science and Human Nutrition and pursued a PhD in Experimental and Clinical Nutrition at the University of Milan. Dr. Del Bo' has been a research fellow at the Department of Food Science and Human Nutrition of the University of Maine (Orono, ME). In addition, he conducted an internship at the Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University (Boston, MA), and at the Department of Public Health of the University of Copenhagen, (Copenhagen, DK).

Del Bò's research focuses on the evaluation of the role of berries, in particular, blueberry, in the modulation of markers related to cardiovascular risk. He performed clinical and animal research documenting the impact of blueberry in the modulation of vascular function and of oxidative stress. Moreover, Del Bò carried out several in vitro studies devoted to the understanding of the role of anthocyanins and metabolic products on inflammatory and atherogenic process. Del Bò is member of the Italian Society of Human Nutrition and of the Groupe Polyphénols. He is on the Editorial Board of the International Journal of Food Sciences and Nutrition.

# Role of Berries and Bioactives in the Modulation of Vascular Function: Evidence from In Vitro and In Vivo Studies

Endothelium is a single cell layer that covers all blood vessels and regulates the passage of macromolecules and circulating cells from blood to tissues [1]. Endothelium is involved in a wide range of factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation [1]. Prolonged and/or repeated exposure to an oxidative stress and inflammatory condition can damage the endothelium which consequently becoming dysfunctional [2-4]. Endothelial dysfunction is considered the primary step in the development of atherosclerosis and cardiovascular disease [5]. This condition is characterized by a reduction of the bioavailability of vasodilators, particularly nitric oxide (NO), and an increase in endothelium-derived contracting factors (e.g. endothelin and vasoconstrictor prostanoids) [5-6].

Berries are an excellent source of bioactive compounds such as vitamins, minerals but above all polyphenols with anthocyanins and phenolic acids as the most representative compounds [7]. Evidence from in vitro and animal models supports the role of these bioactives and their metabolites in the maintenance of normal endothelial cells function thanks to their capability to modulate the expression and activity of several enzymes involved in NO metabolism [8-9]. In addition, polyphenols elicit cell adaptive responses involving the transcription factor Nrf2 and the acute activation of antioxidant and detoxifying enzymes against reactive oxygen species [10]. Furthermore, polyphenol compounds have been shown to reduce pro-inflammatory pathway by inhibiting redox-sensitive transcription factor NF- $\kappa$ B and the expression of a plethora of pro-inflammatory agents and adhesion molecules involved in the bond of monocytes to endothelial cells [11-14].

In this regard, recent studies performed in our laboratories demonstrated the capacity of anthocyanins and phenolic acids to counteract the adhesion of monocytes to endothelial cells also at concentrations comparable to those found in blood following dietary intake [15].

Regarding humans, several studies have reported the effect of (poly)phenols and (poly)phenol-rich foods, including berries, on vascular function [16-20]. Evidence deriving from dietary intervention studies seems promising but deserves more substantiation due to the small amount of existing studies and their wide heterogeneity. Differences in the experimental design, duration of the intervention, type and amount of berry, and markers analyzed, may be the reason for the inconsistencies found in the available data. Another important factor to consider is the type and characteristics of population enrolled. For example, age, sex, dietary habits, physical activity, risk factors and exposure to oxidative stress could contribute to conflicting results present in literature. We performed several acute and chronic interventions devoted to evaluate the effect of blueberry on the modulation of vascular function in healthy volunteers and in subjects with cardiovascular risk factors [21-23]. In this context, we reported that a serving of blueberry purée (300 g providing about 300 mg of anthocyanins) could not improve vascular function in healthy subjects with normal endothelial function [21]. Moreover, six-week intervention with a wild blueberry drink (250 mL providing about 375 mg of anthocyanins) failed to significantly affect vascular function in subjects with cardiovascular risk factors [22]. However, half of the population (i.e. smokers and those with endothelial dysfunction) experienced an improvement of vascular function suggesting that specific risk factors can be critical to underline an effect [22]. Based on these results, we developed an in vivo experimental model to study the vasoactive properties of food/bioactives on endothelial function following a stressor/insult. We showed that a serving of blueberry (300 g providing about 300 mg of anthocyanins) counteracted the temporary impairment of endothelial function, induced by acute cigarette smoke, in a group of smoker volunteers [23]. Moreover, we recently found a significant improvement of vascular function also in smokers and non smokers with an established endothelial dysfunction (data unpublished).

In conclusion, berries and their bioactives seem to play a role in the protection of endothelium. Clearly, robust clinical evidence in humans should be encouraged before a comprehensive understanding of the effects of berries on the modulation of vascular function is achieved.

**Keywords:** Endothelial function; berries; polyphenols, in vitro studies, human intervention studies

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**Joseph Francis, BVSc, MVSc, PhD**  
Louisiana State University

**Joseph Francis, B.V.Sc., M.V.Sc., Ph.D.**, is a veterinarian by profession. He received his Bachelor of Veterinary Medicine in animal husbandry in 1991 and Masters in Veterinary Virology & Immunology in 1994 from Madras Veterinary College, Chennai, India. He then came to the United States to pursue his doctoral studies in neuroendocrinology from Kansas State University, Manhattan, Kansas in 1995. After completion of his doctoral research in 1999, he joined Dr. Robert Felder's lab at the University of Iowa, for a postdoctoral fellowship in cardiovascular pathophysiology. He joined the faculty of Louisiana State University as an Assistant Professor in 2003. He was promoted to Associate Professor in 2007. Currently he is a Professor in comparative biomedical sciences department at Louisiana State University, Baton Rouge, Louisiana. His research interest is on understanding the role played by central nervous system cytokine in the pathophysiology of cardiovascular and renal diseases. More recently he has started working on understanding the role played by brain inflammation in post-traumatic stress disorder. He uses pharmacological and non-pharmacological intervention including blueberries in his research.

## Effects of Blueberries in a Preclinical Model of Post-Traumatic Stress Disorder

Post-Traumatic Stress Disorder (PTSD) is an anxiety disorder that can develop in response to an event that causes psychological trauma. Currently, the diagnosis of PTSD is based on the psychological factors including intrusive recollections of traumatic events, avoidance of stimuli associated with such events, and numbing of general responsiveness not present before the trauma. To date, no definitive diagnostic biomarkers have been identified in PTSD. Recent research, however, points toward physiological changes in the brain and immune system that may be responsible for the psychological manifestations of the disorder. We have established a predator exposure model of PTSD that simulates some of the symptoms of human PTSD. Using this model we have shown that oxidative stress and inflammation is increased in the prefrontal cortex (PFC) and hippocampus (Hippo) of animals with PTSD. We also showed that neurotransmitters are differentially modulated in the brain regions affected in PTSD. Currently, the only approved therapy for PTSD is the selective serotonin re-uptake inhibitors (SSRI), but their efficacy for the treatment of PTSD is marginal. Recently, we demonstrated that over-activation of norepinephrine (NE) along with serotonin (5-HT) as the possible reason for the lack of efficacy of SSRIs. Furthermore, with each passing day, increasing numbers of U.S. combat veterans are diagnosed with PTSD, and suicide rates are rising at an alarming pace. Hence the urgent need for identifying novel molecular target and better approaches for the treatment of PTSD. It is increasingly evident that functional food like blueberries have been shown to attenuate oxidative stress and inflammation. In this presentation, we will examine the role played by blueberries in modulating neurotransmitters system, oxidative stress and inflammation in our preclinical model of PTSD. In addition, PTSD has been related to suicidal thoughts and behavior. The principal origin and the mechanism contributing to the suicidal thoughts in PTSD are not known. Understanding molecular mechanisms and identifying novel genes that might contribute to suicide in the context of PTSD might be vital in the prevention of suicide. Recently, SKA 2 (spindle and kinetochore associated complex subunit 2) a novel genetic and epigenetic target was shown to decrease in patients that attempted or committed suicide. Hence, we will explore, whether our model could be used to study this suicide gene SKA2. Finally, we will determine if blueberries has any role in modulating this gene and protect against stress. Some of our finding might show that blueberries might be an effective non pharmacological additive that could be used to mitigate some of the symptoms/ treatment of PTSD.



**Dr. Arpita Basu**  
Oklahoma State University

Education: PhD, Nutrition & Food Sciences, Texas Woman's University (2005); Postdoctoral training, UC Davis Medical Center; Registered Dietitian (RD); faculty appointment in the Department of Nutritional Sciences, Oklahoma State University (OSU) since 2006. Currently, Associate Professor of Nutritional Sciences, OSU

Awards received: Marguerite Scruggs Award for meritorious early career research in Nutritional Sciences, Oklahoma State University (2009); Best Poster Award (2008 & 2013) American College of Nutrition (ACN) annual conference; Best Poster Award (2005 & 2006) American Society for Nutrition (ASN) annual conference; 58 peer-reviewed original research publications and abstracts.

My teaching and research interests focus on the role of functional foods and phytochemical-based nutraceuticals in reducing risks and complications of diabetes and related cardiovascular conditions. My research group conducts controlled human intervention studies on food and beverages of medicinal health effects such as, green tea, berries, and pomegranate extracts in participants with the metabolic syndrome and type 2 diabetes. We are specifically interested in examining effects on traditional and emerging biomarkers in the clinical progression of diabetes and CVD as modulated by functional foods and phytochemicals.

# Dietary Berries and Osteoarthritis (OA): Effects of Strawberries on Pain and Inflammation in Obese Adults with Radiographic Evidence of Knee OA

The primary focus of my research is to understand the role of dietary sources of bioactive compounds, especially berries in modulating biomarkers of chronic conditions in clinical and epidemiological studies. We have previously reported the effects of whole berry supplementation in improving conventional lipid profiles and size and density-based lipoprotein profiles, and in reducing elevated blood pressure in adults with the metabolic syndrome (1-3). Berries were also shown to selectively modulate biomarkers of lipid peroxidation in these adults. Our findings lie in conformation with other studies that show similar effects of dietary berries in improving serum lipids, and subsequent cardiovascular risks (4). However, possibly due to the target population of intervention being otherwise healthy adults with no cardiovascular 'events', we did not observe any notable effects of berries on markers of inflammation and other cardiometabolic risks. Our study findings support the role of berries in the management of metabolic syndrome and diabetes and associated risks for conditions such as osteoarthritis (OA).

Osteoarthritis (OA), the most common type of arthritis is a chronic, painful and inflammatory musculoskeletal disease affecting 27 million Americans and on the rise with obesity and aging (5). It affects the joints and generates functional impairment. There is no cure for the disease, but some attempts to slow the progression of the disease. Current recommendations for the management of OA combine nonpharmacological and pharmacological interventions, and costly knee replacement procedures. Non-steroidal anti-inflammatory drugs are often associated with gastrointestinal detrimental effects. Therefore, safer alternative interventions are lacking and needed by millions of patients. Nutraceuticals are good candidates for the management of OA due to their safety profile and potential efficacy (6). However, the popularly used supplements such as glucosamine, chondroitin sulfate and avocado-soy unsaponifiables have failed to show improvements of OA symptoms in meta-analysis of randomized clinical trials (7). Commonly consumed functional foods, especially green tea and curcumin show promise in reducing pain and inflammation underlying OA based on preliminary research (8-10). Few human studies have been reported. In our continued efforts to examine the health effects of dietary berries, we conducted a randomized double-blind placebo 28-week controlled crossover trial in obese adults with knee OA. Dietary strawberries showed improvements in pain symptoms associated with knee OA when compared to the control treatment. These results support the emerging role of berries in OA management.

**Keywords:** Osteoarthritis, Metabolic Syndrome, Inflammation, Strawberries, Pain

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**Ting Luo**  
Oregon State University

**Ting Luo**, a PhD candidate in the Food Science and Technology Department at Oregon State University. Her major advisor is Dr. Neil F. Shay, and their collaborative research is focused on the consumption of whole foods and specific phytochemicals and the remediation of metabolic syndrome symptoms. In particular, the approach utilizes a mouse model in which animals are fed a high-saturated fat diet with added cholesterol and sugar to model the Western diet, contributing to the development of obesity, diabetes, and other symptoms of metabolic syndrome. Additionally, their research examines gene- phytochemical interactions and includes metabolomics approaches.

Ms. Luo received her master's degree at the State Key Laboratory of Food Science and Technology, Nanchang University in China, one of China's top programs in Food Science and Nutrition. Her previous research explored the effects of dietary lipids on the occurrence of atherosclerosis; more specifically, the impact of triolein and trilinolein on oxidized low-density lipoprotein-induced oxidative stress in endothelial cells. She is an active member of the American Society for Nutrition, and the American Chemical Society, and has received a graduate student research award from ASN and a teaching award from OSU. Her studies at OSU are being supported in part by a graduate fellowship awarded by the Chinese Scholarship Council. Ting's current research includes investigations on the effect of consumption of phytochemical-rich red raspberries, omega-3- rich English walnuts, and soy isoflavones. In particular, the work investigates impacts on remediation of symptoms of metabolic disease, and on the gene expression regulation via the activation of hepatic transcription factors including the PPARs, LXR, and HNF-4 .

## Consumption of Red Raspberries, at Typical Levels of Intake Reduces Metabolic Syndrome Parameters in High-fat fed Mice

Using an animal model for diet-induced metabolic disease, we have shown previously that the addition of raspberry juice concentrate (RJC, at 10% of kcal) and raspberry puree concentrate (RPC, at 10% of kcal) to an obesigenic Western-style diet (WD) containing high levels of saturated fat, cholesterol, and sugar, significantly reduced body weight gain and serum resistin, a marker correlating with glucose intolerance and diabetes. Compared to the WD-fed mice, expression of hepatic genes related to lipid metabolism and oxidative stress were significantly altered in RJC- and RPC-fed mice (Luo et al., 2016).

As these diets containing 10% of energy from raspberry products represent about four food servings per day, we wished to examine the effect of consumption of a lower level of these polyphenol-rich food products. In this second study, we formulated diets with RJC and RPC included at a level representing a single serving of food per day (2.5%, w/w), a level of intake that humans could typically consume. For ten weeks, four groups of C57BL/6J mice (n=8 ea.) were fed the control and experimental diets: low fat (LF), Western diet (WD), WD+RJC, and WD+RPC. In week nine, a glucose tolerance test was conducted to assess insulin sensitivity and glucose control. After ten weeks, animals were sacrificed, serum collected, and liver tissue saved for histology, lipid accumulation measurements, and gene expression analyses.

When added to a high-fat, Western-style diet at the equivalent of a single serving per day, RJC and RPC decreased final body weight and overall weight gain. While there was a slight decrease in baseline blood glucose concentrations, and more significant decreases in serum insulin levels, the area under the curve in a glucose tolerance test showed no differences. Liver and adipose tissue weights were consistent with improved metabolism with both RPC and RJC intake compared to consumption of the obesigenic high-fat, high-calorie diet. The relative expression of genes associated with oxidative stress (Glutathione S-transferase alpha 1, *Gsta1*; Heme oxygenase 1, *HMOX-1*) were altered by diet, as well as one gene associated with lipid metabolism (Hormone sensitive lipase, *HSL*).

In this animal model, it appears that consumption of the equivalent of a single daily serving of either RPC or RJC improves metabolism in mice fed the high-fat, Western-style diet. We hypothesize that the phytochemicals contained in raspberries, and/or their subsequent metabolites, may be acting to influence gene expression and other regulatory pathways, to produce the metabolic improvements observed in this study.

**Keywords:** Raspberry, Metabolic disease, Glucose metabolism, Oxidative stress, Lipid metabolism

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**Wednesday, March 29th - Scientific Presentations**



## **Berries and Brain Aging**



**Dr. Barbara Shukitt-Hale**  
USDA & Tufts University

Session Chair

Current Research Review

**Dr. Barbara Shukitt-Hale** is a USDA Staff Scientist in the Laboratory of Neuroscience and Aging, USDA-ARS, Human Nutrition Research Center on Aging (HNRCA) at Tufts University in Boston, MA. Additionally, she serves as an Affiliate Faculty member in the Psychology Department and a Visiting Scholar in the Friedman School of Nutrition Science and Policy at Tufts University. She received her Ph.D. in Experimental Psychology from Boston University in 1993.

In 1996, Dr. Shukitt-Hale was awarded the Glenn Post-Doctoral Award, presented by the American Aging Association. She is a member of the Society for Neuroscience and has served as a board member and secretary of the American Aging Association. Dr. Shukitt-Hale has been involved in research for almost 30 years, beginning when she was an undergraduate student at Boston University; this work earned her the Research Award, given at graduation to the best student researcher in the Psychology Department. Before coming to the HNRCA, she worked as a Research Psychologist in the Division of Health and Performance and as a Neuroscientist in the Military Performance and Neuroscience Division at the U.S. Army Research Institute of Environmental Medicine (USARIEM).

Dr. Shukitt-Hale's current work involves researching the behavioral and neurochemical effects of aging in rodents, specifically investigating motor and cognitive performance changes due to oxidative stress, using the free-radical theory of aging as a working model. Her work includes determining the factors responsible for age-related behavioral changes and possible amelioration of these effects with various nutritional treatments. Her work showing that a diet supplemented with blueberry extract could reverse functional age-related deficits in motor and cognitive behavior has had a tremendous impact in the popular press. She continues to research the mechanisms behind the berry fruit's positive effects, and has found that they 1) have direct effects on signaling to enhance neuronal communication, 2) have the ability to buffer against excess calcium, 3) enhance neuroprotective stress shock proteins, and 4) reduce stress signals and increase neurogenesis. She has published more than 166 articles and selected papers.



**Amanda N. Carey, PhD**  
Simmons College

**Amanda N. Carey, Ph.D.** is an Assistant Professor in the Department of Psychology at Simmons College in Boston, MA, USA.

She received her Ph.D. in Psychology from Northeastern University in 2010. Her doctoral research was funded by an NRSA training grant and examined the effects of the HIV viral protein Tat on the brain and behavior using a transgenic mouse model. Specifically, she elucidated the role of Tat protein in the mediation of cognitive deficits associated with a neurodegenerative condition called NeuroAIDS. Also prior to her academic appointment at

Simmons, Dr. Carey was a Postdoctoral Research Affiliate at Tufts Human Nutrition Research Center on Aging (HNRCA). She studied how nutrition can modulate age-related behavioral and neurochemical changes. She is continuing to investigate the effects of diet on the brain and behavior in her research laboratory at Simmons College. Dr. Carey's current research examines how a high fat diet can affect behavior and brain functioning and the cognitive and neurological benefits of eating high antioxidant berries. However, these two research tracks are not mutually exclusive. She is engaged in projects investigating if blueberry and raspberry supplementation of a high fat diet can prevent impairments in spatial memory and novel object recognition. She is also researching if supplementation with berries can increase plasticity in the brains of mice fed high fat diet.

She is presently the PI on an industry-sponsored award investigating the ability of red raspberry supplementation to prevent or allay the cognitive, neurological and metabolic alterations induced by consumption of a high fat diet.

Dr. Carey spends a significant amount of her time mentoring and involving undergraduates in her research. She has authored over 25 publications that have been cited over 100 times and is on the editorial board for the journal *Nutritional Neuroscience*. In her free time she likes to Irish dance.

## Mitigating the Effects of High Fat Diet in the Brain with Berry Supplementation

Obesity is a major public health concern that affects more than one third of adults in the US.<sup>1</sup> Research suggests that consuming a high-energy high-fat diet (HFD) increases inflammation and oxidative stress and that these changes often parallel some of the brain alterations associated with aging.<sup>3-5</sup> However, evidence is accumulating that consuming berries can prevent and even reverse the neurochemical and behavioral changes associated with aging.<sup>6-9</sup> Thus, it stands to reason that berries may confer similar benefit in those consuming a HFD. Our research suggests that both blueberries and raspberries are able to mitigate some of the behavioral alterations associated with HFD consumption in a mouse model of obesity.

In one study<sup>10</sup>, middle-aged C57Bl/6 mice were fed low-fat diet (LFD) or HFD (60% calories from fat) with and without 4% freeze-dried blueberry (U.S. Highbush Blueberry Council). There was a reduction in memory impairment in the mice that consumed HFD supplemented with 4% freeze-dried blueberry compared to HFD-fed mice without blueberry supplementation. Novel object recognition learning and memory was tested after 2, 3, and 4 months on the diets. Mice fed HFD showed recognition memory deficits at all time points, but mice fed HFD + blueberry showed a reversal of memory disruption at 4 months. Furthermore, probe trial performance in the Morris water maze was impaired in animals consuming HFD, while animals on HFD + blueberry performed similarly to those on LFD. Following behavioral testing,<sup>10</sup> brain-derived neurotrophic factor (BDNF) and neurogenesis was measured in the brains of the mice in order to assess if blueberry could enhance neuroplasticity in mice fed HFD. Neuroplasticity is the brain's ability to compensate for injury and disease and to adjust in response to changes in the environment. BDNF levels and neurogenesis were enhanced in the brains of mice fed HFD + blueberry. These increases were observed in the hippocampus, which is an area of the brain important for the creation of memories. This neuroplasticity may underlie the reduced behavioral impairment in the middle-aged mice fed HFD diet supplemented with blueberry.

In a subsequent study, young mice were fed similar diets, but instead were supplemented with 4% freeze-dried raspberry powder (Van Drunen Farms). The combination of the large presence of ellagitannins and high levels of anthocyanins and vitamin C suggests that raspberries have the potential for great health-promoting effects. After 5 months on the diets, mice were tested in the Barnes maze, which tests the ability of mice to learn and remember the location of an escape from a brightly lit maze. Mice fed HFD + raspberry performed significantly better than the HFD-fed mice, suggesting that raspberry is protecting the mice from the effects that HFD consumption has on cognition. We also found that raspberry supplementation prevented HFD-associated elevations in the inflammatory cytokine interleukin-6 in the brain. This reduction in brain inflammation may be responsible, at least in part, for the preserved Barnes maze performance of the HFD + raspberry-fed mice.

These studies demonstrate that both blueberry and raspberry may provide a level of protection against the effects that HFD can have on one's brain. However, additional research using human subjects will be needed to confirm this.

**Keywords:** berry supplementation, blueberry, brain, high fat diet, neuroplasticity, raspberry

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**Dr. Claire Williams**  
University of Reading, UK

**Professor Williams** is Chair of Neuroscience in the School of Psychology & Clinical Language Sciences at the University of Reading, UK.

She received her PhD in Psychology from the University of Reading in 2000. Her research group, the Nutritional Psychology laboratory, investigates the health benefits of plant-derived chemicals. The main focus of her laboratory is the interplay between dietary intake and measures of psychological well-being such as cognitive performance, food preference, mood, and quality of life using a wide range of techniques (e.g. animal studies, randomised controlled trials, neuroimaging) and population groups (e.g., school-aged children, healthy adults, older adults, patients with mild cognitive impairment). The group have published a number of articles including a demonstration that improvements in spatial working memory induced by a high flavanoid diet can be linked to de novo protein synthesis in rat hippocampus, flavonoid supplementation is associated with increased cerebral blood perfusion in healthy older adults, and that single acute doses of blueberries can significantly improve memory and attention in children aged 8-10 years old.

Professor Williams is currently PI on an industry-sponsored award investigating the effects of an anthocyanin-rich supplement on cognitive performance in 65-80 year olds and is Co-I on a 3-year UK Research Council funded grant investigating the mechanisms underlying the acute and chronic cognitive effects of flavanol/anthocyanin intervention in humans. She has published more than forty peer-reviewed research articles, four book chapters and four patents.

# Effects of Flavonoid-Rich Blueberries on Cognitive Function in Healthy Younger and Older Adults

**Authors:** Claire M. Williams<sup>1</sup>, Georgina F. Dodd<sup>1,2</sup>, Daniel J. Lamport<sup>1</sup>, Jeremy P.E. Spencer<sup>2</sup> & Laurie T. Butler<sup>1</sup>

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The increase in incidence and prevalence of neurodegenerative diseases, along with an absence of effective drug treatments, highlights the need for a more comprehensive understanding of how different aspects of lifestyle, such as exercise and diet, may affect neural function and consequently cognitive performance. In particular, flavonoids, found in a variety of fruits, vegetables and beverages, have been recognized as promising agents capable of influencing different aspects of synaptic plasticity resulting in improvements in memory and learning in both animals and humans (Williams & Spencer, 2012; Del Rio et al, 2013). A large body of evidence has emerged from human intervention studies demonstrating that the consumption of flavonoid-rich foods is associated with cognitive benefits (for reviews Macready et al, 2010; Lamport et al, 2012).

Here, we report two acute cross-over randomised controlled trials to investigate the effects of flavonoid-rich blueberry beverages on cognitive function in adults, and the mechanisms by which they may be producing these effects. Healthy younger and older adults received either a flavonoid-rich blueberry beverage, or matched placebo, with performance on a cognitive battery measured at baseline, two and five hours post-treatment. Stiffness index (SI), blood pressure (BP) and levels of brain-derived neurotrophic factor (BDNF) were recorded at baseline and one hour. Whilst there was no effect of the blueberry beverage on SI, BP or global cognitive function, the blueberry beverage did result in improved executive function in the young adults and memory in the older adults. In both groups, BDNF levels were higher following consumption of the blueberry beverage compared to placebo. In a follow-up study in healthy younger adults, functional magnetic resonance imaging (fMRI) was conducted to determine whether the blueberry intervention resulted in increased cerebral blood flow (CBF). No effects of the blueberry intervention were seen on whole brain or gray matter CBF, however there was a greater CBF in areas of precentral and middle frontal gyrus, as well as the angular gyrus, following the blueberry compared to placebo drink.

In conclusion, acute blueberry supplementation led to improvement in results in cognitive benefits mediated by an action on BDNF signalling pathways, in addition to vasodilatory properties and subsequent CBF increases. These results demonstrate how important a diet rich in berries may be for health, particularly in relation to counteracting the decline in human cognitive function associated with normal and abnormal ageing.

**Keywords:** Flavonoid, Anthocyanins, Blueberry, Cognition, Memory, Executive Function

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## **Pamela Maher, PhD**

**Salk Institute**

**Pamela Maher** is at the Salk Institute for Biological Studies in La Jolla, California. She has an undergraduate degree in biochemistry from McGill University and a PhD in biochemistry from the University of British Columbia. She did her postdoctoral work with Jon Singer at the University of California, San Diego. After helping to establish a research program at the Whittier Institute in La Jolla with Nobel Laureate Roger Guillemin, she held a faculty position at Scripps Research Institute before joining the Salk Institute in 2004.

As a postdoctoral fellow, she was instrumental in the development of the technology used for the detection of phosphorylated proteins involved in cell signaling pathways, opening up a whole new field of cell biology. She then used these methods to discover several novel mechanisms for the transfer of information within cells.

Dr. Maher has also published extensively in the area of growth factors in the nervous system, and on the basis of this work identified several natural products that mimic the effects of protein growth factors in the nervous system. Since protein growth factors are of limited use for the treatment of brain diseases because they cannot get into the brain, the identification of small molecules that readily enter the brain and mimic the effects of protein growth factors provides a new therapeutic avenue for the treatment of nervous system diseases such as Alzheimer's.

Her recent work has focused on identifying additional natural products that are effective against brain diseases, improving the natural products already identified using medicinal chemistry and characterizing their molecular targets so as to provide additional approaches to the treatment of brain diseases. She has over 125 peer-reviewed publications and is currently supported by both public and private funding agencies.

# The Berry Flavonoid Fisetin is Protective in Multiple Animal Models of Age-Associated Neurological Disorders

In addition to some well known flavonoids such as quercetin, berries contain several less well known flavonoids including the flavonol fisetin (3,7,3',4' tetrahydroxyflavone) which has been reported to be especially abundant in strawberries (1). We have found that fisetin is an orally active, novel neuroprotective and cognition-enhancing molecule (2). Fisetin was originally identified in a screen for compounds that could prevent oxidative stress-induced nerve cell death (3). Of the ~30 flavonoids and related polyphenols tested in this study only two, fisetin and quercetin, were able to maintain GSH levels in the presence of oxidative stress, indicating that this is not a common property of flavonoids and related polyphenols. Further screens with many additional flavonoids and related polyphenols have confirmed this observation. We focused on flavonoids and related polyphenols in this screen because they were known to have a number of activities that could be beneficial for the maintenance of CNS function. Further studies showed that fisetin also possessed neurotrophic activity, promoting the differentiation of PC12 cells via activation of the Ras-ERK cascade (4). Again, this was a property that distinguished fisetin from almost all of the other ~30 flavonoids tested. Together, these observations suggested that fisetin had multiple properties that might make it useful for the treatment of age-related neurological diseases. Further studies have demonstrated that fisetin can protect nerve cells from multiple toxic insults including amyloid toxicity, ischemia and hyperglycemia. It has both direct antioxidant activity and maintains the levels of GSH under conditions of stress by inducing the transcription factors, Nrf2 and ATF4 (5). Fisetin is also able to maintain ATP levels under ischemic conditions (6). Moreover, fisetin was shown to facilitate long term potentiation (LTP) in hippocampal slices via modulation of ERK and CREB phosphorylation and oral administration of fisetin promoted learning and memory in mice using the object recognition test (7). Fisetin is also effective in two different models of stroke (6,8), at least partly through its anti-inflammatory effects. Using three different models of Huntington's disease (mutant huntingtin-expressing PC12 cells, mutant huntingtin-expressing *Drosophila* and the R6/2 mouse) we found that fisetin was able to reduce the impact of mutant huntingtin in each of these disease models (9). In a genetic model of type 1 diabetes, fisetin reduced diabetic complications in both the kidney and brain (10). Fisetin also prevents learning and memory deficits and neuroinflammation in APP<sup>sw</sup>/PS1<sup>dE9</sup> (huAPP/PS1) double transgenic Alzheimer's disease (AD) mice (11). More recently, using rapidly aging SAMP8 mice, a model of sporadic AD and dementia, fisetin was found to reduce cognitive deficits while restoring multiple markers associated with impaired synaptic function, stress and inflammation. Finally, fisetin was found to reduce dopamine loss in the MPTP model of Parkinson's disease.

Unlike many polyphenols, fisetin has reasonable ADME properties and active metabolites have a long plasma half life (12,13) and reach high levels in the cerebrospinal fluid. For example, we recently determined that sulfated and/or glucuronidated forms of fisetin reach concentrations of 30  $\mu$ M in the cerebrospinal fluid with a plasma half life of 8 hr in macaques fed a single oral dose at 25 mg/kg. Moreover, using label free two photon microscopy, we recently showed that fisetin localizes to the brain parenchyma after oral administration (14). Fisetin is apparently safe based on both short-term toxicity studies and our long-term animal studies (11). In addition, it is currently commercially available in the nutraceutical market.

**Keywords:** Alzheimer's disease, glutathione, neuroinflammation, cognition, diabetes

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**Robert Krikorian** is Professor in the Department of Psychiatry & Behavioral Neuroscience and Director of the Cognitive Aging Program at the University of Cincinnati Academic Health Center. His clinical and research interests include the influence of health conditions on memory decline and risk for Alzheimer's disease and non-pharmaceutical interventions to forestall progression of neurodegeneration. His current research involves investigations of the effects of flavonoid supplementation and macronutrient manipulation on neurocognitive function in middle-aged and older adults. Funding for his research has come from the National Institutes of Health and from foundation and industry sources.

# Berry Fruit Supplementation in Cognitive Aging: Advances in Human Berry Trials

There is an impressive body of evidence from preclinical research demonstrating benefits of berry fruit supplementation for cognitive performance and aspects of brain function, particularly with respect to the effects of blueberry consumption introduced in aging animals (1). On the other hand, much less berry research concerning human neurocognitive function has been completed to date. However, controlled human trials are increasing in number and studies have been extended to investigate brain function in addition to assessing cognitive performance. Further, recent data concerning human metabolism of anthocyanin compounds provide information that is essential for understanding more about the mechanisms that underlie cognitive benefits (2).

We will review the findings of our human trials examining the effects of berry supplementation on cognitive performance and brain function in older adults (3-5). Recently, our studies have incorporated novel data on anthocyanin intake in the background diet as well as measures of food form anthocyanins and their metabolites (6). These recent trials have demonstrated a number of interesting findings. We have shown that cognitive benefits associated with blueberry supplementation are more prominent in older adults with greater impairment. In addition, berry fruit supplementation can enhance neural response to challenging cognitive tasks, although apparently not in association with improved performance on such tasks. Further, diet record analyses and assays of parent anthocyanins and metabolites indicate that older, human research participants can maintain prohibitions against consumption of anthocyanin-containing foods in the background diet relatively well (6). More importantly, these data, in conjunction with the evidence of cognitive benefit in blueberry-supplemented individuals, suggest that cognitive and neural function enhancements are related more specifically to the acute effects of food-form anthocyanins as opposed to the effects of the extended metabolism of the parent compounds.

Generally, these studies and most other human berry intervention trials are subject to methodological limitations and uncertainties, in particular associated with dose and duration of supplementation, flavonoid consumption external to the research intervention, uncertainty about metabolic processes and mechanisms, and small sample size. Ultimately, larger sample sizes will be essential to establish more robust findings concerning neurocognitive benefits. This will be necessary not only to increase statistical power but also to mitigate effects of individual difference factors such as variability in anthocyanin absorption and metabolism and in response to their biological effects. Further, heterogeneity in cognitive capability independent of age and neurodegenerative factors can influence results in small sample studies.

Nonetheless, recent investigations with blueberries and other berry fruits continue to demonstrate neurocognitive benefit and suggest that supplementation may provide significant risk reduction when practiced in advance of dementia. This work is particularly salient in a time when emphasis on preventive measures and risk mitigation is increasing and pharmaceutical trials have not provided hope of mitigating or reversing cognitive decline after the onset of late-life dementia.

**Keywords:** blueberries; anthocyanins; aging; memory; brain activation

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**Thursday, March 30th - Scientific Presentations**



## **Berries and Cancer**



**Dr. Ramesh Gupta**  
University of Louisville

Session Chair

Current Research Review

**Dr. Ramesh C. Gupta** received PhD in Chemistry from the Roorkee University (now Indian Institute of Technology), India, and then moved to Baylor College of Medicine, Houston for postdoctoral training in 1973. He grew to Associate Professor at Baylor prior to moving to University of Kentucky in 1989 as Professor. In 2003, he was recruited by James Graham Brown Cancer Center, University of Louisville and was appointed as Professor, Distinguished University Scholar and Agnes Brown Duggan Chair in Oncological Research. He has always worked at the cutting edge technology pioneering sensitive methods to sequence tRNAs, followed by ultrasensitive  $^{32}\text{P}$ -postlabeling to measure DNA damage by environmental carcinogens. These works have received several thousand citations.

Last year, Dr. Gupta's laboratory reported the development of novel polymeric implants for continuously ("24/7") delivering natural compounds for long duration for prevention and treatment of cancer. This technology has been filed for patents by the University of Louisville - part of the patent issued in March 2012. His recent focus has been to identify natural compounds and extracts which attack multiple targets for preventing lung, breast and cervical cancers. His laboratory was the first to report the inhibition of breast cancer and lung cancer by blueberry 'colored' compounds, and cervical cancer by withaferin A isolated from the ancient Indian herb "ashwagandha". The blueberry compounds have also elicited enhanced response of chemotherapeutic drugs which led to a lung cancer clinical trial.

The laboratory's thrust is to develop simple and effective strategies for prevention and treatment of cancer recurrence and metastasis using blueberry bioactives and other natural compounds and novel drug delivery systems. He has been fortunate to have a qualified team of researchers, continuous funding from NIH and State grants, the Duggan endowment and James Graham Brown Cancer Center.



**Farrukh Aqil, PhD**  
University of Louisville

**Farrukh Aqil, Ph.D.**, is an Assistant Professor of Medicine at the James Graham Brown Cancer Center, University of Louisville. He received his doctorate in Microbiology from Aligarh Muslim University, India and has extensive experience of over 14 years in phytochemistry, microbiology, cancer biology and cancer prevention. Prior to moving to the United States as a Postdoctoral Associate in the Cancer Chemoprevention Group, he has a faculty in the Department of Biotechnology, Integral University, India.

His research focus is on cancer chemoprevention primarily of breast, lung and ovarian cancers using both standard chemotherapeutic drugs and agents from natural origin like berries. In the last few years, his focus has been to evaluate the effectiveness of selected berries against lung cancer. In another project, he has focused on chemopreventive efficacy and mechanisms of whole-berry and spice powder against breast cancer. He has developed analytical techniques for tissue and plasma distribution of bioactive principles. Finally, he is developing novel combinatorial approaches for the treatment of lung and ovarian cancer by testing natural agents and standard chemo drugs using drug-sensitive and drug-resistant cancer cells.

More recently, he has played a key role in the development of polymeric implants for continuous systemic and local delivery of drugs, a technology which has fetched several patents. Another upcoming drug delivery technology in which he has also played a key role is based on biocompatible exosomes for delivery of small molecules and siRNAs. He showed that exosomes can deliver chemopreventives and chemotherapeutics effectively. He has participated in many conferences and presented his work in the form of 75 abstracts/oral presentations. Dr. Aqil has authored or co-authored over 55 articles in peer-reviewed journals, has 13 book chapters and has edited 4 books. He is an associate editor for the International Research Journal of Microbiology and serves as peer reviewer for more than 30 journals.

# Berry Anthos for the Management of Various Cancers

**Authors:** Farrukh Aqil<sup>1,2</sup>, Jeyaprakash Jeyabalan<sup>1</sup>, Ashish K. Agrawal<sup>1</sup>, Lynn Parker<sup>3</sup>, Radha Munagala<sup>1,2</sup> and Ramesh C. Gupta<sup>1,4</sup>

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Berry bioactives, particularly the colored pigments, anthocyanins/anthocyanidins are known for their antioxidant, anti-proliferative, apoptotic and anti-inflammatory properties. Data from our and other laboratories indicate preventive and therapeutic activities of berries and berry bioactives against various cancer types. We have also demonstrated that anthocyanidins (Anthos) isolated from berries have significantly higher antiproliferative activity versus their glycons (i.e., anthocyanins), and that exosomal formulation of Anthos have enhanced anti-proliferative, anti-inflammatory and anti-cancer activity against various cancers (lung, breast, ovarian, pancreatic cancer, etc). Data discussed in this report is focused against ovarian cancer.

We examined the antiproliferative activity of bilberry-derived Anthos against drug-sensitive (A2780) and drug-resistant (A2780/CP70, OVCA432 and OVCA433) ovarian cancer cells. These OVCA cell lines are PgP-overexpressing and show >100-fold resistance to chemotherapeutic drug cisplatin (Cis-Pt) versus the drug-sensitive cells (A2780). We observed dose-dependent inhibition of all cell lines with the Anthos. Further, Anthos (75  $\mu$ M) in combination with Cis-Pt showed significantly higher kill (10-15 fold lower IC50) of the drug-resistant ovarian cancer (OVCA 432) cells compared with Cis-Pt alone. This combination regimen also showed enhanced inhibition of the other ovarian cancer cells.

However, many plant bioactives including Anthos face the challenge of poor oral bioavailability; Anthos also encounter stability issue. Recently, we have developed new strategies using bovine milk-derived exosomes to enhance efficacy of berry Anthos. The Anthos when encapsulated in the milk-derived exosomes significantly enhanced antiproliferative activity (>10-20 fold) and/or reduced dose against the growth of various cancer types including ovarian cancer in vitro. Antitumor activity of the Anthos was determined in a nude mice inoculated subcutaneously with the A2780 ovarian cancer cells and the exosomal formulation was delivered by oral gavage. Measurement of tumor volume showed significant growth inhibition with the exosomal-Anthos (65%) compared to vehicle control. Significantly higher antitumor activity was also found when the Anthos formulation was combined with paclitaxel, a widely used chemotherapeutic drug. Finally, no systemic toxicity was observed following treatment of wild-type mice with the exosomes. Together, our data demonstrate that the Anthos can be developed as a potent therapeutic agent to kill cancer cells.

**Keywords:** Blueberries, Anthocyanidins, Anthocyanins, Exosome, Drug delivery, Anti-cancer activity

(Supported from Agnes Brown Duggan Endowment and Helmsley Trust Funds)



**Chantal Matar, PhD, RD**  
University of Ottawa

**Dr. Chantal Matar** obtained her Ph.D. in Food Sciences and Technology from Laval University, Canada (1997) and a Dietetic Internship from Ottawa Hospital (2010). Her expertise is focused on in vivo assessing of functional foods (probiotic, bioactive peptides, and polyphenol-enriched nutraceutical preparations) in immunosurveillance, anti-inflammatory response and chemoprevention of cancer by controlling cancer stem cells and microRNAs. In collaboration with the WHO's International Agency for Research on Cancer, she established a prevention-based research program on nutrition and neoplasia mechanisms. She successfully managed and led active research lab in biomedical, nutrition/immunology/cancer. She is an established investigator with proven track record of supervising highly qualified personnel. She authored more than 110 communications, including 40 referred papers and book chapters, and 4 patent applications.

She is particularly involved in the research on nutrition and health, probiotics, microbiome and chemoprevention of breast and skin cancer by functional foods and was successful in acquiring research funding from different research agencies.

She is maintaining strong collaborations at both national and international levels, as evidenced by international awards: 1) Best Research Award from Trade and Industry Ministry (Japan) for a new study presented at the 24th and 22nd International Congress on Nutrition and Integrative Medicine held in Sapporo, Japan 2014, 2) Life Member of the Association of International Union Against Cancer Fellows and Visiting Scientist at WHO 2009, and 3) Scientific Advisor for the International Life Sciences Institute North America Canadian Advisory Committee 2016.

# Mechanisms of Chemoprevention of Breast Cancer by Biofermented Blueberry Preparation: An Interface Between Nutrition and Cancer

Our prevention-based research program on nutrition/cancer is aimed to analyse molecular and signalling pathways involved in the neoplasia mechanisms. We have reported that mammary carcinoma in mice are prevented by orally administered Polyphenol-Enriched Blueberry Preparation (PEBP) following fermentation by a probiotic-like novel bacterium through anti-inflammatory mechanisms, and enhanced immunosurveillance. Probiotic-like product -PEBP- showed higher, biofunctionality and antioxidant activity compared to its non-fermented counterpart [1, 2]. Blueberry polyphenols are known as effective chemopreventative compounds for breast cancer [3], as well as influencing oncogenic signaling pathways and re-sensitization of drug resistant cancer cells [4]. Probiotic fermentation of blueberry contribute to the conversion of blueberry polyphenols to smaller oligomers with higher bioavailability, thus enhancing their therapeutic value. In collaboration with the International Agency for Research on Cancer, we have shown that PEBP supports a diet-mediated targeting of Cancer Stem Cells by significantly inhibiting the metastasis in an in vivo model. Cancer Stem cells are a subpopulation of highly tumorigenic cells population that are driving tumor resistance. Epigenetic modulation of Cancer Stem Cells is an important feature in controlling their development at the level of methylation or miRNA expression [5, 6].

Our research is focused on understanding the role of probiotic fermented blueberry in influencing signalling pathways and miRNA signature. We are currently evaluating their role in tertiary prevention to re-sensitize chemoresistance breast Cancer Stem Cells to chemotherapeutic drugs. In the same line, we are studying their role in modulating chemoresistance-induced miRNAs. We aim to effectively provide novel evidence-based data for the usage of fermented berries in chemoprevention as well as for reducing cancer resistance and improving therapeutic index and patient survival.

**Keywords:** Probiotic/blueberry, Chemoprevention, Re-sensitization, Cancer Stem Cells, miRNAs

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**Jeevan K. Prasain, PhD**  
University of Alabama

**Jeevan K. Prasain, PhD** is an Assistant Professor at the Department of Pharmacology & Toxicology, University of Alabama at Birmingham. His current research interest includes lipidomics/metabolomics, bioactive dietary natural products in the prevention of chronic diseases such as diabetes and cancers, their metabolisms, and bioavailability assessment using liquid chromatography tandem mass spectrometry (LC-MS/MS).

# Cranberry Fruits in Bladder Cancer Prevention

Authors: Jeevan K. Prasain<sup>1</sup>, Rajani Rajbhandari<sup>1</sup>, Adam B. Keeton<sup>2</sup>, Gary A. Piazza<sup>2</sup>, Clinton Grubbs<sup>3</sup>, Stephen Barnes<sup>1</sup>

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Bladder cancer is one of the commonly diagnosed malignancies in both males and females in industrialized countries and large bodies of epidemiological studies have shown that consumption of fruits and vegetables is inversely associated with the incidence of bladder cancer. Particularly, a role of dietary phytochemicals such as polyphenols in the prevention of urinary bladder carcinogenesis is feasible since most substances and/or their metabolites are excreted through the urinary tract in relatively high amounts.

My previous collaborative study with Dr. Clinton Grubbs (Department of Surgery, UAB) on the role of cranberry juice in bladder cancer prevention in animals demonstrated that a commercially available cranberry juice concentrate can prevent urinary bladder cancers dose-dependently in N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN) induced bladder carcinogenesis in rats when compared to the control group (Prasain et al., 2008). Our studies further indicated that cranberry polyphenols such as quercetin, once ingested, reach to the urinary bladder and concentrate in the urine either intact or in conjugated metabolite forms, and may be associated with prevention of carcinogenesis (Prasain et al., 2008). Based on these results, cranberry juice may be an effective cancer chemopreventive agent for bladder cancer and its metabolites quercetin and methylquercetin stored in urine and bladder tissue protect against the progression of carcinogenesis (Rajbhandari et al., 2011).

To identify urinary metabolites of cranberry juice responsible for the growth inhibition of bladder cancer, anti-proliferative activities of number of polyphenols were evaluated using a panel of bladder cancer cell lines (RT4, SCABER, SV-HUC1, and SW-780). Among the compounds tested, quercetin 3-O-glucoside, isorhamnetin (3'-O-methylquercetin), myricetin and quercetin showed strong concentration-dependent cell growth inhibitory activities in bladder cancer cells with IC50 values in a range of 8-92  $\mu$ M (Prasain et al., 2016). We next attempted to determine whether the differential cell growth inhibitory effects of isomeric quercetin 3-O-glucoside (active) and quercetin 3-O-galactoside (inactive) are related to their metabolism by the cancer cells. For this, SW-780 cells were incubated with these compounds and their metabolites were examined by LC-MS/MS. Compared to quercetin 3-O-glucoside, quercetin 3-O-galactoside undergoes relatively less metabolic biotransformation. These results suggest that cranberry fruits represent a reservoir of phytochemicals with potential anti-cancer properties, and emphasize the importance of diets rich in cranberries in bladder cancer prevention.

**Keywords:** Cranberry, quercetin, LC-MS/MS, metabolism, bladder cancer

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A black and white photograph of raspberries, showing the texture of the fruit and the leaves. The raspberries are in the foreground, and the background is blurred.

**Thursday, March 30th - Scientific Presentations**

**Berries and Gut Health/Gut Microflora**



**Dr. Jess Reed**  
University of Wisconsin-Madison  
Session Chair  
Current Research Review

**Dr. Jess Reed** is Professor of Animal Nutrition at the University of Wisconsin-Madison. He received a PhD from Cornell in 1983. His 33 years of research has focused on the effects of phytochemicals in foods and forages on human and animal health and nutrition, including 6 years at the International Livestock Center for Africa where he studied the phytochemistry of tropical legume forages.

Starting in 1996, he began researching the effects of flavonoids in foods on human health, including cardiovascular disease, urinary tract infections and cancer. Reed has over 100 research publications in his field and a successful research program funded through competitive grants from NIH and USDA along with collaborative projects with the food and nutritional supplements industry. Dr. Reed also maintains an active outreach program in agricultural development with project experience in 20 countries.



**Li-Shu Wang, PhD**  
Medical College of Wisconsin

**Dr. Wang** is an Associate Professor of Medicine, Medical College of Wisconsin. Dr. Wang's research interests are in the prevention of cancers using nature products and their active metabolites. She has experience in evaluating the effects of chemopreventive agents, including black raspberries, on gene expression in vitro (in mammary and colon cell culture systems) and in vivo (in the rat esophagus, colon cancer and pancreatic cancer).

Using bio-directed fractionation, she showed that the anthocyanins in black raspberries are important for their chemopreventive effects and she provided evidence that the ellagitannins may be less important. Recently, she has evidence that berries cause demethylation of tumor suppressor genes in rodent and human colon leading to their enhanced expression in two human clinical trials. The protective effects of berries against human and mouse colorectal cancers are associated, at least in part, with their hypomethylation activities. Loss of responses to berry treatment in humans may be due to decreased sensitivity to berry-induced DNA demethylation. Dr. Wang's recent findings suggest that berries produced beneficial effects against colonic adenoma development in a mouse colon cancer model and modulated multiple metabolic pathways. Similarly, black raspberry intervention induced significant metabolic changes and affected energy generating pathways in human colon cancer patients.

# The Axis of Gut Bacteria-Metabolites-Their Receptors in Colon Carcinogenesis

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Our body is host to trillions of microbes, and they connect with and affect our host system (1,2). Gut dysbiosis associates with many diseases, including colorectal cancer (3). Some natural compounds and foodstuff could alter the diversity and composition of gut microbiome, potentially benefiting our health (4). Our previous studies demonstrated that black raspberries (BRBs) are chemopreventive against colorectal cancer (5-8). To investigate the effects of whole BRBs and their fiber fraction on gut microbiota, we fed F-344 rats a control AIN-76A diet or the control diet supplemented with 5% BRBs or the fiber fraction from 5% BRBs for 6 weeks. Feces were collected at the baseline and at weeks 3 and 6, and bacterial sequencing was analyzed using 16S rRNA. We observed distinct patterns of gut microbiota after different diets. Beta diversity analysis showed that baseline (week 0) samples were segregated from post-treatment (weeks 3 and 6) samples within each dietary group, suggesting that both the whole BRBs and the fiber fraction induced time-dependent changes in the bacterial diversity. Gut bacteria can ferment dietary fibers into short-chain fatty acids (SCFAs), which activate free fatty acid receptor 2 (FFAR2) (9,10). We showed that BRBs suppressed the proliferation of colon cancer cells by activating FFAR2, whereas loss of FFAR2 signaling promoted colon carcinogenesis in *ApcMin/+* mice, a mouse model of colon cancer (11). Thus, we further investigated if FFAR2 signaling affects the gut microbiome and bacteria-produced metabolites. Principal coordinate analyses (PCA) showed different clusters between *ApcMin/+* and *ApcMin/+*-FFAR2<sup>-/-</sup> mice. Relative abundance of bacteria at family level showed increased levels of Bacteroidaceae, Sphingobacteriaceae and Porphyromonadaceae, and decreased levels of Ruminococcaceae and Bifidobacteriaceae in both *ApcMin/+* mice and *ApcMin/+*-FFAR2<sup>-/-</sup> mice compared to wild-type (WT) mice. Decreased Bifidobacterium has been reported in human colon cancer tissues (12), consistent with our results in mice. More importantly, we observed that the levels of Flavobacteriaceae and Verrucomicrobiaceae were further increased in *ApcMin/+*-FFAR2<sup>-/-</sup> mice compared with *ApcMin/+* mice, which could contribute to FFAR2 deficiency-promoted colon carcinogenesis in *ApcMin/+*-FFAR2<sup>-/-</sup> mice. In addition, gut bacteria could deconjugate a significant portion of the primary bile acids, and structurally modify them into secondary bile acids, which have been shown to promote colon carcinogenesis (13). We observed significantly increased levels of secondary bile acids, including deoxycholate, 6-beta-hydroxylithocholate, and 7-ketolithocholate in *ApcMin/+*-FFAR2<sup>-/-</sup> mice compared to *ApcMin/+* mice. Deoxycholate has been demonstrated to promote colon carcinogenesis by 165.1% in *ApcMin/+* mice (14), suggesting that increased secondary bile acids could directly contribute to FFAR2 deficiency-promoted colon cancer development. Collectively, our results suggest that BRBs are capable of changing homeostasis of gut bacteria. Our results also suggest the importance of the receptors to the metabolites in maintaining the gut bacteria homeostasis. All together, we suggest the axis of gut bacteria-metabolites-receptors can greatly influence colon carcinogenesis.

**Keywords:** black raspberries, fiber, gut microbiota, metabolomics, bile acids

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**Dr. Chris Gill**  
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PhD from Ulster University (2000). Senior lecturer (2014) in nutrition at the Ulster University and thematic leader for “Phytochemicals and gut health” within the Northern

Ireland Centre for Food and Health (NICHE). His research focuses on the influence of diet on gut health investigated through in vitro, animal and human studies. Dr Gill has published 30+ research papers, 6 book chapters and 1 patent. He has developed an extensive research network with internationally leading research centres and secured significant research income of £1.6 million from a range of sources from prestigious sources including the EU, NPRC and FIRM. Editor for the European Journal of Nutrition (2014) and recent recipient of the University of Ulster Distinguished Research Fellowship (2015). Dr Gill has also acted as a member of the Scientific Advisory Board and expert panel member for Scandinavian Nutrition funding programmes since 2008.

# Raspberry Phytochemicals are Bioactive Following in Vivo Digestion

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The anticancer properties of bioactive phytochemicals within berries are of interest given the inverse correlation of fruit and vegetable consumption with the incidence of colorectal cancer (CRC)<sup>1</sup>. Berries are one of the most commonly consumed sources of polyphenols and these compounds may exert protective effects against initiation of CRC by reducing DNA damage<sup>2</sup>.

The purpose of the study was to assess the bioactivity of raspberry phytochemicals identified in vivo with respect to markers of gut health. Ileal fluid was collected from eleven ileostomates, pre and post consumption of red raspberries (300g) and assessed for phytochemical composition by LCMSn<sup>3</sup>. Targeted approaches identified major anthocyanin and ellagitannin components at varying recoveries and with considerable inter-individual variation. Non-targeted LC-MSn analysis identified novel raspberry-specific metabolites. Some components (including ellagitannin and previously unidentified proanthocyanidin derivatives) may have arisen from raspberry seeds that survived intact in ileal samples. We putatively identified the novel component as a fruit triterpenoid glycoside which had an apparent MW of 680 and could be purified from raspberry seeds (triterpenoid-enriched fraction-TRF)<sup>4</sup>.

We then simulated the interaction of the colonic microbiota and the ileal fluids (pre and post berry consumption) using 24 h in vitro gut fermentation modelling, which resulted in a significant increase in the presence of simple phenolic compound(s) including benzoic acid (9 of 11 participants) and 3-(3'-hydroxyphenyl) propionic acid (10 of 11 participants).

The post-berry ileal fermentate from 9 of the 11 ileostomates significantly decreased DNA damage (~40%, P<0.05) in the CCD841 CON normal cell line using an oxidative challenge COMET assay (25 µm H<sub>2</sub>O<sub>2</sub>); this protective effect was not observed for pre-berry ileal fermentate samples. Furthermore, when we isolated our novel raspberry triterpenoid (TRF) and tested it using the above model, significant (P<0.05) anti-genotoxicity was observed at 100 nM.

Gene expression studies using real-time PCR demonstrated that the ileal fermentates and the TRF modulated the expression of genes of the Nrf2-ARE pathway involved in oxidative stress cytoprotection<sup>5</sup>, namely nuclear factor (erythroid-derived 2)-like 2 (Nrf2), NAD(P)H Dehydrogenase, Quinone-1 (NQO1) and Heme oxygenase-1 (HO-1).

To conclude, following consumption of raspberries, phytochemicals survive digestion in the gut and likely enter the colon in vivo. We have demonstrated that the physiologically relevant berry enriched ileal fermentate and a novel raspberry triterpenoid (TRF) identified from ileal fluid reduce DNA damage in normal colonocytes which may be mediated in part by the Nrf2-ARE pathway.

**Keywords:** Raspberries, Ileostomy, DNA Damage, Digestion, In vitro fermentation

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This work was supported by a grant from the National Processed Raspberry Council and the Scottish Government's RESAS department.



## **Amy Howell, PhD** Rutgers University

**Dr. Amy B. Howell** is an associate research scientist at the Marucci Center for Blueberry and Cranberry Research at Rutgers University, where she works on isolating natural products from cranberries that benefit health.

Since 1993, Dr. Howell has been engaged in research aimed at identifying the active compounds in cranberries that prevent urinary tract infections and determining their role in maintenance of urinary tract health. Dr. Howell and her team isolated specific compounds from cranberry fruit, called proanthocyanidins (PACs), which they found to be capable of preventing *E. coli* bacteria from attaching to cells from the urinary tract. This work was published in *The New England Journal of Medicine* in 1998.

In a subsequent publication in *The Journal of the American Medical Association*, she reported on cranberry's potential role in preventing antibiotic-resistant bacteria from colonizing the urinary tract. Her work on identification of the unique molecular structures of the A-type cranberry PACs has been published in both *Phytochemistry* and the *Journal of Natural Products*.

Currently, she is engaged in projects to determine additional mechanisms of action in the gut for cranberry and maintenance of urinary tract health. She is closely involved in method development for accurate quantification of cranberry PACs in powdered supplements to enable the cranberry industry to develop, manufacture and market high quality, efficacious products for human and animal nutrition. She is currently involved in writing the USP monographs on cranberry, and serves on the AOAC SPSFAM Proanthocyanidins in Cranberries (PAC) Working Group.

She has presented her research findings at numerous professional meetings in the U.S. and internationally, and her work has been featured in magazines and newspapers (*NY Times*, etc.) and has been a guest on radio and television shows (*NPR*, *Dr. Oz*, *Today Show*, *Good Morning America*, etc.)

## Ulcers, Stomach Cancer, Antibiotic Resistance and Cranberries: What's the Connection?

*Helicobacter pylori* (*H. pylori*) is one of the most important bacteria world-wide with prevalence rates of over 80% in some developing countries (1). About two-thirds of the world is infected with *H. pylori*. In the U.S., it is more prevalent among older adults, African Americans, Hispanics, and lower socioeconomic groups. Infection with *H. pylori* is associated with development of most gastric ulcers and, if untreated, increases the risk of developing stomach cancer (2). Infected individuals have a 2- to 6-fold increased risk of developing gastric cancer and mucosal-associated-lymphoid-type lymphoma. Gastric cancer is the second most prevalent cancer worldwide, common in countries such as China, Chile and Colombia, where *H. pylori* infect children early in childhood. Treatments to eradicate the bacteria can be quite harsh and involve triple or quadruple antibiotic therapy, which has numerous side-effects and can promote selection for antibiotic resistant strains of *H. pylori* (3). There is also about a 20% failure rate for triple therapy. In many countries, it is difficult and expensive to obtain treatment, so the bacteria persist and increase the risk of developing stomach cancer in the future. To cause ulcers, *H. pylori* penetrate through the mucus layer of the stomach and adhere to the underlying epithelial layer. As the mucus layer breaks down, acid attacks the stomach lining and causes an ulcer. One of the adhesins that mediates attachment of the bacteria to the epithelium is similar to that found on P-fimbriated *Escherichia coli* (*E. coli*) (4) one of the major uropathogenic bacteria that cause urinary tract infections. Cranberry proanthocyanidins inhibit adhesion of P-fimbriated *E. coli* to uroepithelial cells and have been demonstrated to prevent adhesion of some strains of *H. pylori* to human mucosal cells (5).

In a clinical study with patients receiving triple antibiotic therapy for *H. pylori* infection, ingestion of cranberry juice drink (250 ml/day) combined with the antibiotic treatment improved rate of eradication in females (6). Another clinical study in China demonstrated that consumption of two 250-ml servings of cranberry juice cocktail (27% cranberry) per day resulted in a 15% eradication of *H. pylori* (7). A third clinical trial that took place in Chile found a 200-ml serving of cranberry juice or *Lactobacillus* probiotic for three weeks inhibited *H. pylori* in 15% of asymptomatic children (8). Given that antibiotic resistance rates to traditional antibiotic regimens to treat *H. pylori* are increasing worldwide, and the triple or quadruple antibiotics that are prescribed can be quite deleterious to patients and have a high failure rate, cranberry-based products could be a potentially viable alternative strategy to fight ulcers, thereby reducing the incidence of stomach cancer.

**Keywords:** *Helicobacter pylori*, stomach ulcers, stomach cancer, cranberry proanthocyanidins, anti-adhesion, antibiotic resistance

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## **Dr. Laura Kresty**

### **Medical College of Wisconsin**

**Laura Kresty, Ph.D., M.S.** is an Associate Professor of Medicine at the Medical College of Wisconsin Division of Hematology and Oncology, specializing in Cancer Prevention. Dr. Kresty received her PhD in Public Health from The Ohio State University in 2000 with a major in Cancer Chemoprevention and Epidemiology and minor in Health Promotion and Disease Prevention. Dr. Kresty remained at The Ohio State University to complete a NCI-Sponsored Post-Doctoral Fellowship in Molecular Oncology, followed by a faculty appointment in Internal Medicine.

In 2008 Dr. Kresty joined the University of Miami, Miller School of Medicine and Sylvester Cancer where she continued her research focused on the inhibition of esophageal adenocarcinoma and cancers of the head and neck. Dr. Kresty also served as Director for the Doctorate in Epidemiology Program and was a Cancer Biology Steering Committee Member at the University of Miami.

In January 2013, Dr. Kresty joined the Medical College of Wisconsin where she continues research investigating risk factors, molecular mechanisms and preventive strategies for targeting cancers of the esophagus and head and neck. Dr. Kresty serves as an Associate Editor for *Molecular Carcinogenesis*, is a peer reviewer for multiple additional journals in her field, has over 50 peer-reviewed research articles and book chapters and has delivered 45 invited talks throughout the world.

In addition, she is a Standing Member of NCI's Chemo/Dietary Prevention Study Section and frequently serves on other NIH/NCI review and special emphasis panels. Her ongoing research focus is on evaluating the cancer inhibitory potential of various dietary constituents, including cranberry proanthocyanidins (C-PACs).

Her research team has reported for the first time that C-PAC activates autophagic cell machinery leading to cell death, the specific type of C-PAC-induced cell death depends on sensitivity to bile acids, C-PAC-induced death is Beclin-1 independent and importantly Beclin-1 inactivation is linked to esophageal adenocarcinoma stage and grade. Dr. Kresty is also interested in energy excess as it relates to escalating esophageal adenocarcinoma risk and collaborating on investigations focused on novel imaging technologies to detect early epithelial and sub-epithelial esophageal changes for more rapid evaluation of chemopreventive agents.

# Cranberry Proanthocyanidins Reverse Microbial Dysbiosis and Inhibit Bile Acid Metabolism in Association with Esophageal Cancer Prevention

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Our laboratory has been investigating the cancer inhibitory potential of cranberry proanthocyanidins (C-PAC) against esophageal adenocarcinoma (EAC), a cancer characterized by rapidly rising incident rates and poor survival (1-2). The 5-year survival rate for those diagnosed with EAC, all stages combined, is consistently less than 18% (1). Utilizing a panel of validated human esophageal cancer cell lines and EAC xenograft bearing mice, our laboratory reported for the first time that C-PAC activates autophagic cellular processes leading to cancer cell death which is largely caspase-independent; yet linked to bile acid sensitivity (3,4). Reflux of bile and stomach acid into the lower esophagus is considered the major risk factor for progression to EAC. The widespread use of endoscopy and treatment with anti-reflux medications has only modestly impacted this rising and deadly malignancy. Thus, there is an urgent need for new approaches to counteract reflux-induced alterations in key cancer-related signaling pathways.

Building on our initial research (3-9), we next investigated additional mechanisms by which C-PAC inhibits reflux-induced EAC with a focus on gut microbiome alterations and modulation of bacterial metabolites. Cranberry extracts have previously been reported to reduce urinary tract infections by inhibiting bacterial adhesion, colonization and movement (10,11). Moreover, research shows a shift in the bacterial species colonizing the esophagus of patients with acid-reflux and EAC (12); yet, the impact of cranberries has not been evaluated in this context. In addition, primary bile acids undergo secondary metabolism by bacteria in the intestinal tract forming secondary bile acids and therefore it is plausible that alterations in bacterial profiles will in turn alter the bile acid composition and exposure to injurious refluxant. We utilized the rat surgical esophagogastrroduodenal anastomosis (EGDA) model to mimic human reflux-induced EAC and investigated the potential cancer inhibitory mechanisms associated with orally delivered C-PAC. Reflux/EGDA<sup>+</sup> and non-surgical Sprague Dawley rats were treated with water or C-PAC (690 µg/rat/day) for 25 or 40 weeks and assessed for cancer progression via extensive histopathological and molecular characterization. Additionally, at 40 weeks, fecal microbiome profiling was investigated and global metabolic profiling conducted on esophageal, liver and fecal samples. Methods included 16s rRNA sequencing of rat fecal DNA, paired end sequencing on Illumina MiSeq and data analysis using Qiime and the R packages phyloseq and edgeR to assess C-PAC-induced microbiome changes. For metabolite profiling homogenized esophagi, liver and fecal samples were extracted in methanol and characterized by Reverse Phase Ultra high Performance Liquid Chromatography-Tandem Mass Spectrometry RP/UPLC-MS/MS, followed by metabolite identification based on Metabolon's library of authenticated standards.

Results support that C-PAC significantly inhibits the formation of EAC with concomitant restoration of the normal gut microbial profile, i.e., the bacterial profile shifted toward increased favorable Gram positive Firmicutes and away from inflammatory-linked Gram negative Bacteroidetes and Proteobacteria. A number of microbiome-derived or microbiome-contributed biochemicals were significantly altered in Reflux/EGDA<sup>+</sup> animals with C-PAC treatment reversing the deleterious effects. C-PAC significantly reduced primary and secondary bile acid metabolites in the esophagus of reflux/EGDA<sup>+</sup> rats supporting mitigation of an important risk factor for EAC progression. Other bacterial-linked metabolites significantly modulated by C-PAC included derivatives of histidine, lysine, phenylalanine, tyrosine and tryptophan. C-PAC also significantly reduced a number of pro-inflammatory eicosanoids and additional lipid metabolites.

In summary, these results support that (1) reflux-induced microbial impairment correlates with the stimulation of bile acid metabolism and (2) C-PAC mitigates reflux-induced microbial and metabolite changes, inflammation and injury in the esophagus in association with EAC inhibition. Future research focused on mechanisms by which C-PAC restores dysbiosis and favorably impacts metabolic profiles in the esophagus is warranted to inform clinical trials in patients with gastroesophageal reflux disease. Consideration should be given to C-PACs temporal effects to determine whether microbiome and metabolite changes are causal in EAC progression; bile receptor mediated effects, bile acid transport and transcriptional regulation following C-PAC treatment; bacterial adherence/colonization/motility as well as bacterial challenge studies with specific bile acids alone and in combination with C-PAC and investigations of C-PACs capacity to mitigate bile-induced DNA damage.

**Keywords:** Cranberry proanthocyanidins (C-PAC), Barrett's esophagus, esophageal adenocarcinoma, cancer prevention, dysbiosis, microbiome, metabolic profiling, bile acid and microbial metabolites.

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# Poster Presentation Abstracts





**Luke R. Howard, PhD**  
University of Arkansas  
Poster Session Chair

**Dr. Howard** received his B.S. degree in Horticulture from Purdue University, and his M.S. and Ph.D. degrees in Food Science from the University of Arkansas. He worked as an Analytical Chemist at the Dole Packaged Foods Research and Development Center for two years, and was an Assistant Professor in the Horticultural Sciences Department at Texas A&M University for five years. He has served on the faculty in the Department of Food Science at the University of Arkansas since 1997 (Associate Research Professor 1997-2002, Professor 2002-present). His research program is focused on extraction and characterization of bioactive compounds in fresh and processed fruits and vegetables, with emphasis on berries. Dr. Howard has published over 120 scientific articles and five book chapters and has delivered over 90 presentations at scientific meetings. He is a Professional Member of the American Chemical Society.

# Nutraceutical Evaluation of Two Wild Berries (*Vaccinium Consanguineum* and *Ugni myricoides*) Collected From the Vicinity of Irazú Volcano, Costa Rica

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Berries constitute one of the most significant sources of potential health-supporting phytochemicals in the human diet. They are rich in nutraceuticals components such as proanthocyanidins (PACs), phenolic acids, carotenoids and others.

The objective of this research consisted of an assessment in the content of phenolic and proanthocyanidins compounds, also to measure of the antibiotic and antioxidant activity present in the crude extract of two berries collected in the foothills of the Irazú volcano, Cartago-Costa Rica. The parts of the plant used in this research were fruit (ripe and unripe), leaves and stem. Columns packed with C-18 and Sephadex LH-20 resins were used to obtain standards of polyphenols and proanthocyanidins, respectively. The content of total phenolics in the crude extract of *V. consanguineum* using the Folin-Ciocalteu method showed  $146 \pm 5$  mg of gallic Acid eq/g of dry sample for the leaves,  $98 \pm 1$  for the stem and  $24.8 \pm 0.5$  for both ripe and unripe fruit. On the other hand, the *U. myricoides* results were lower,  $84.5 \pm 0.4$  mg of gallic Acid eq/g of dry sample for the leaves,  $40.8 \pm 0.1$  for the stem,  $91 \pm 4$  for the unripe fruit and  $69 \pm 2$  for the ripe fruit. The concentration of PACs using the DMAC method showed the following results; *V. consanguineum* gave  $30.5 \pm 0.5$  mg catechin eq. (CE)/g of dry sample for the leaves,  $38.1 \pm 0.5$  for the stem,  $5.54 \pm 0.07$  for the ripe fruit and  $12.4 \pm 0.5$  for the unripe fruit. The *U. myricoides* samples showed  $31 \pm 3$  for the leaves,  $24 \pm 4$  for the stem,  $11.6 \pm 0.9$  for the ripe fruit and  $15.4 \pm 0.2$  for the unripe fruit. The antioxidant activity was determined using the ORAC method. The *V. consanguineum* results were of  $69941 \pm 5876$   $\mu$ M Trolox eq/g of dry sample for leaves,  $62,617 \pm 1463$  for the stems,  $23,912 \pm 739$  for the ripe fruit and  $32,503 \pm 3111$  for the unripe fruit. For the *U. myricoides* samples, the ORAC results were of  $52,241 \pm 3106$  for the leaves,  $23,025 \pm 2647$  for the stems,  $38,339 \pm 7773$  for the ripe fruit and  $45,053 \pm 3728$  for the unripe fruit.

Finally, the antibacterial activities of two types of extracts (crude extract of polyphenols (CEP) and crude extract of proanthocyanidins (CEPACs), 3 mg) of *V. consanguineum* and *U. miricoides* were tested against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* using the disc diffusion method. Percentage of zones of inhibition of the extracts were compared with those of a standard antibiotic (chloramphenicol  $1\mu$ g, 100%) to determine the antibacterial activities. The average percentage (%) of growth inhibitions of all the extracts were  $92 \pm 3$ ,  $62 \pm 3$ ,  $62 \pm 4$  and  $68 \pm 3\%$  against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*, respectively. In general, the percentage of growth bacterial inhibitions was slightly higher with the CEPACs in comparison with the CEP, while the *Ugni* extracts were a little more effective inhibiting the bacterial growth than *Vaccinium* extracts.

# The Axis of Gut Bacteria-Metabolites-Receptors in Colon Carcinogenesis

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Our body is host to trillions of microbes, and they connect with and affect our host system. Gut dysbiosis associates with many diseases, including colorectal cancer. Some natural compounds and foodstuff could alter the diversity and composition of gut microbiome, potentially benefiting our health. Our previous studies demonstrated that black raspberries (BRBs) are chemopreventive against colorectal cancer. To investigate the effects of whole BRBs and their fiber fraction on gut microbiota, we fed F-344 rats a control AIN-76A diet or the control diet supplemented with 5% BRBs or the fiber fraction from 5% BRBs for 6 weeks. Feces were collected at the baseline and at weeks 3 and 6, and bacterial sequencing was analyzed using 16S rRNA. We observed distinct patterns of gut microbiota after different diets. Beta diversity analysis showed that baseline (week 0) samples were segregated from post-treatment (weeks 3 and 6) samples within each dietary group, suggesting that both the whole BRBs and the fiber fraction induced time-dependent changes in the bacterial diversity. Gut bacteria can ferment dietary fibers into short-chain fatty acids (SCFAs), which activate free fatty acid receptor 2 (FFAR2). We showed that BRBs suppressed the proliferation of colon cancer cells by activating FFAR2, whereas loss of FFAR2 signaling promoted colon carcinogenesis in *ApcMin/+* mice, a mouse model of colon cancer. Thus, we further investigated if FFAR2 signaling affects the gut microbiome and bacteria-produced metabolites. Principal coordinate analyses (PCA) showed different clusters between *ApcMin/+* and *ApcMin/+FFAR2-/-* mice. Relative abundance of bacteria at family level showed increased levels of Bacteroidaceae, Sphingobacteriaceae and Porphyromonadaceae, and decreased levels of Ruminococcaceae and Bifidobacteriaceae in both *ApcMin/+* mice and *ApcMin/+FFAR2-/-* mice compared to wild-type (WT) mice. Decreased Bifidobacterium has been reported in human colon cancer tissues, consistent with our results in mice. More importantly, we observed that the levels of Flavobacteriaceae and Verrucomicrobiaceae were further increased in *ApcMin/+FFAR2-/-* mice compared with *ApcMin/+* mice, which could contribute to FFAR2 deficiency-promoted colon carcinogenesis in *ApcMin/+FFAR2-/-* mice. In addition, gut bacteria could deconjugate a significant portion of the primary bile acids, and structurally modify them into secondary bile acids, which have been shown to promote colon carcinogenesis. We observed significantly increased levels of secondary bile acids, including deoxycholate, 6-beta-hydroxylithocholate, and 7-ketolithocholate in *ApcMin/+FFAR2-/-* mice compared to *ApcMin/+* mice. Deoxycholate has been demonstrated to promote colon carcinogenesis by 165.1% in *ApcMin/+* mice, suggesting that increased secondary bile acids could directly contribute to FFAR2 deficiency-promoted colon cancer development. Collectively, our results suggest that BRBs are capable of changing homeostasis of gut bacteria. Our results also suggest the importance of the receptors to the metabolites in maintaining the gut bacteria homeostasis. All together, we suggest the axis of gut bacteria-metabolites-receptors can greatly influence colon carcinogenesis.

## Neuroprotective Effects of Anthocyanin-Enriched Extracts of Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry and Strawberry

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The accumulation of advanced glycation end products (AGEs) leads to inflammatory responses via the receptor of AGEs (RAGE) and to neurodegenerative diseases such as Alzheimer's disease (AD). Our group has recently reported that anthocyanin-enriched extracts (ACEs) from six common edible berries inhibited the formation of advanced glycation endproducts (AGEs). As part of our continued research interest in identifying dietary agents for AD prevention, herein, we evaluated the neuroprotective effects of ACEs purified from six common edible berries, namely, blackberry (*Rubus* spp.), black raspberry (*Rubus occidentalis*), blueberry (*Vaccinium angustifolium*), cranberry (*Vaccinium macrocarpon*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*). The ACEs were evaluated for their inhibitory effects of the acetylcholinesterase enzyme (AChE) which catalyzes the breakdown of neurotransmitters including acetylcholine and plays an important role in the development of AD. The ACEs were evaluated in a microplate assay and compared to galanthamine, a known AChE inhibitor (63.8 % inhibition at 10 µg/mL). At concentrations of 100 µg/mL, the ACEs of blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry showed anti-AChE activity ranging from 27-47%. In addition, cyanidin-3-glucoside, a major anthocyanin in several of these ACEs, also showed potent activity of 51.3% inhibition at 100 µM. This study suggests that anthocyanins are among major contributors to the anti-AChE activities of these common edible berries. Our group is currently evaluating the anti-neuroinflammatory effects of the berry ACEs in murine BV-2 microglia cells in vitro.

## Identifying the Role of Fruit Phytochemicals in Modulating Allergen-Induced Lung Inflammation

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It is estimated that 150 million people are affected by asthma worldwide, with a 5-15% prevalence in children. Numerous epidemiological studies have shown that certain bioactive compounds, including anthocyanins and proanthocyanins, are associated with the attenuation of lung inflammation. We studied the effects of the phytochemical composition of blackcurrant and boysenberry fruits on mechanisms of protection against ovalbumin (OVA)-induced lung inflammation in mice. Our research determined that the different phytochemical profiles of these berryfruit have different effects on the cellular processes related to lung inflammation. This suggests that these mechanistic differences of berryfruits can be exploited to create functional fruit-based foods to manage airway inflammation.

# Prevention and Treatment of Colorectal Cancer by Bilberry-Derived Anthocyanidins (Anthos) and Nano-Anthos

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Familial adenomatous polyposis (FAP) is an inherited disorder caused by mutations in the adenomatous polyposis coli (APC) gene. If left untreated, nearly 100% of patients with FAP will eventually develop colorectal cancer, which is the third most common form of cancer diagnosed in men and women and the third leading class for cancer-related deaths within the United States. Given the prevalence of colon cancer both in the United States and worldwide, further insight into developing novel and more effective prevention and treatment strategies are warranted. A wide variety of plant-derived compounds have been invaluable sources of medicines throughout history. The family of plant pigments known as the anthocyanins has been identified with a variety of health benefits including chemopreventive as well as therapeutic effects due to their roles as anti-inflammatory and antioxidant agents. Found in dark-colored fruits, especially berries, vegetables, grains and flowers, anthocyanins provide the characteristic hues to blueberry, blackberries, strawberries, eggplant, black rice and hibiscus flowers. However, a limitation to current clinical applications of anthocyanins is the high doses that are required due to their poor stability and bioavailability. Recent work within our group has shown that milk-derived exosomes can significantly increase bioavailability and efficacy of plant bioactives compared with the free compounds. We hypothesized that bilberry Anthos (non-glycosylated anthocyanins) are more effective than the native bilberry anthocyanins, and that nano formulation of the Anthos (ExoAnthos), prepared by embedding Anthos onto milk-derived exosomes, could enhance therapeutic efficacy compared with free Anthos for colorectal cancer prevention and treatment. The antiproliferative effects of Anthos and ExoAnthos against APC mutant (HT-29) and APC wild-type (HCT116) colon cancer cell lines were assessed using an MTT assay. Native mixture of Anthos were isolated from commercially available enriched bilberry extract by acid hydrolysis followed by solvent extraction and further purified by C18 column chromatography. To assess chemopreventive effects, the impact of the Anthos on polyp number was investigated in the APCMin/+ mouse model for FAP. Therapeutic efficacy of Anthos against colorectal cancer was assessed using an APCMin/+ Enterotoxigenic *Bacteroides fragilis* mouse tumor model. Results from antiproliferation studies showed that ExoAnthos significantly lowered the IC50 compared with the free Anthos against colon cancer cells. Studies in an APCMin/+ mouse model for FAP showed a significant reduction in polyp number in the Anthos-treated mice. Furthermore, preliminary studies in the APCMin/+ colon cancer mouse model showed that treatment with the Anthos led to a significant reduction in tumor number. Together, these data provide a promising outlook on the future of the berry Anthos for the prevention and treatment of colorectal cancer. (Work supported from the Agnes Brown Duggan Endowment, Helmsley Trust Fund, R21AI092133 and IPIBS fellowship (to AM)).

# Phenolic Compounds from Wild Blueberry (*Vaccinium angustifolium*) inhibit Complement Activation by Targeting C1s-mediated Cleavage of C4

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Current research indicates that ingestion of berries containing polyphenols, such as flavonoids, phenolic acids and anthocyanins are associated with lower risk of inflammatory, metabolic, cardiovascular and degenerative diseases. Diet has been shown to modulate the activation of the complement system, a set of over 50 proteins present in the circulation and tissues that reacts in response to damage or microbial encounter and is critical for the maintenance of body homeostasis. Imbalanced activation of the complement system is tightly correlated with inflammation and various pathologies. Wild blueberries (*Vaccinium angustifolium*) are a rich source of anthocyanins and other phenolic compounds, which can be found in the plasma shortly after consumption. Given the involvement of both complement and polyphenols in the modulation of inflammation, we set to investigate whether wild blueberries modulate the activation of the complement system. Phenolic- and anthocyanin-rich fractions were extracted from freeze-dried wild blueberry powder (Cherryfield, Maine, USA), were characterized by liquid chromatography (Alliance mod. 2695, Water, Milford, MA) and used in *in vitro* complement inhibition assays. Here, we document that both phenolic and anthocyanin fractions inhibit the activation of the complement classical pathway in a dose-dependent manner with IC<sub>50</sub> of 325.6µg/ml and 605.6µg/ml respectively. The activation of the alternative complement pathway was not impacted by the presence of the bioactives. Individual anthocyanins malvidin and cyanidin, as well as the phenolic metabolites syringic acid, protocatechuric acid, gallic acid, chlorogenic acid, and hippuric acid also showed complement inhibitory activity with an IC<sub>50</sub> of approximately, 1mM. Mechanistically, we determined that polyphenols impact specifically the complement classical pathway by targeting the activation of the complement protein C4 through the C1s enzyme. This study presents novel data on the inhibition of the complement classical pathway by phenolic compounds extracted from wild blueberries, shedding new light on the anti-inflammatory properties of berries and potential health benefits of berry consumption.

# The Role of Anthocyanin and Phenolic-Rich Fractions from Wild Blueberries (*Vaccinium Angustifolium*) on Endothelial Cell

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Angiogenesis is a normal biological process that occurs in tissue development and is highly linked to wound healing and a plethora of pathological conditions such as atherosclerosis. This study investigates the effect of anthocyanin (ACNs) and phenolic acid (PA)-rich fractions and their combination from wild blueberry powder (*Vaccinium angustifolium*) on endothelial cell migration related to angiogenesis. The objectives are to study whether ACNs, PAs and their combinations affect: a. Proliferation rate of the endothelial cells and b. Speed of endothelial cell migration after acute exposure to different concentrations of ACNs, PAs and their combinations.

Human umbilical vein endothelial cells (HUV-EC-C [HUVEC] (ATCC® CRL-1730™)) were used and the AlamarBlue cytotoxicity assay was performed to determine the appropriate ACN and PA concentrations for the cell migration experiments. ACNs and PA-rich fractions were extracted from freeze dried wild blueberry powder (Cherryfield, Maine, USA) and characterized by liquid chromatography (Alliance mod. 2695, Water, Milford, MA). Anthocyanins (0.0001µg/ml - 1000µg/ml) and PAs (0.0001µg/ml - 500µg/ml) were tested to determine their cytotoxicity after 24h exposure. The speed of endothelial cell migration (µm/hour) was measured by live-cell imaging (Nikon TS100) with usage of the wound healing assay dish (Ibidi, Munich, Germany). Anthocyanins at 0.002µg/ml, 8µg/ml, 15µg/ml, 60µg/ml, 120µg/ml and 300µg/ml, PAs at 0.002µg/ml, 8µg/ml, 15µg/ml, 60µg/ml, 120µg/ml and 300µg/ml and combination of both bioactive compounds at 0.002µg/ml, 8µg/ml, 15µg/ml, 60µg/ml, 120µg/ml and 300µg/ml from each ACN and PA-rich fraction were tested after exposure of HUVECs for a maximum of fifteen (15) hours. Cytotoxicity assays documented that ACNs at 1000µg/ml was toxic to HUVECs and was not used in further experiments. Analysis of the time-lapse videos (TScratch, Zurich, Switzerland) documented inhibition of endothelial cell migration speed (µm/hour) when cells were treated with 60µg/ml of ACNs (28.3µm/hour) compared to control (34.3µm/hour) (p<0.05). In contrast to ACNs, PAs at 0.002µg/ml, 60 µg/ml and 120µg/ml increased migration speed (47.06µm/hour, 45.7µm/hour and 40.36µm/hour respectively) compared to control (34.3µm/hour) (p<0.05). Moreover, combination of both compounds at concentrations of 8µg/ml: 8µg/ml (ACNs:PAs) and 60 µg/ml: 60µg/ml (ACNs:PAs) revealed increased migration speed (39.98µm/hour and 46.09µm/hour respectively) compared to control (34.3µm/hour) (p<0.05).

Findings suggest that endothelial cell migration is differentially modulated based on the bioactive fraction and is concentration-dependent. ACNs appear to inhibit HUVEC migration while PAs promote this process. Further investigation is necessary to determine the mechanisms behind this biological phenomenon with possible implications to atherosclerosis.

# Cranberries Modulate Postprandial Glucose and Inflammation Following a High-Fat Breakfast Challenge in Adults with Type 2 Diabetes

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**Background:** Recent research supports a favorable role of cranberries on measures of cardiometabolic health, including serum lipid profiles, blood pressure, endothelial function, glucoregulation, as well as biomarkers of inflammation and oxidative stress. Postprandial metabolism, especially hyperglycemia has been shown to be an independent cardiovascular risk and few clinical studies have reported on the role of berries in improving postprandial dysmetabolism. **Purpose:** We investigated the postprandial effects of dried cranberries following a high-fat breakfast challenge in obese participants with type 2 diabetes (T2DM), in a randomized crossover trial. **Methods:** Blood draws and vascular measurements were conducted at fasting, 1, 2 and 4 hours (h), following consumption of a fast-food style high-fat breakfast (50g fat, 766 kcal). Serum glucose and lipids were analyzed using standard clinical chemistry. Biomarkers of inflammation were determined using ELISA techniques.

**Results:** Analyses of our data (n=25; BMI (kg/m<sup>2</sup>) (mean±SD) =39.5±6.5; Age (years) =56±6) revealed that postprandial changes in glucose were significantly lower in the cranberry vs. control at 2 & 4h (p<0.05). No significant differences were noted in lipid profiles between cranberry and control groups. Among the biomarkers of inflammation, serum interleukin-18 (IL-18) tended to be lower at 2h (p<0.1) and was significantly lower at 4h (p<0.05) in the cranberry vs. control group. No effects were noted in postprandial changes in C-reactive protein and interleukin-6 (IL-6). Among vascular parameters, no significant differences were noted in systolic and diastolic blood pressure, as well as in small and large artery elasticity indices. **Conclusions:** Overall, dietary cranberries were shown to exert notable effects on high-fat breakfast induced postprandial glucose and selected biomarkers of inflammation in participants with T2DM. These findings define the role of whole cranberries in postprandial blood glucose management and warrant further investigation.

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## Stabilization of Anthocyanins in Blackberry Juice

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Blackberry anthocyanins provide a vivid attractive color and antioxidant activity; however, anthocyanins degrade during juice processing and storage. Maintaining high anthocyanin concentrations in berry juices may lead to greater antioxidant and health benefits for the consumer. The objective of this study was to evaluate potential additives to stabilize anthocyanins during storage of blackberry juice. The anthocyanin stabilizing agents; glutathione, galacturonic acid, diethylenetriaminepentaacetic acid and tannic acid were added to blackberry juice at a concentration of 500 mg/L. Juices were evaluated over five weeks of accelerated storage at 40°C for anthocyanin flavonol and ellagitannin contents by HPLC and percent polymeric color. Glutathione had the greatest protective effect with 11% greater retention ( $p=0.04$ ) of total anthocyanins and 21.5% less ( $p<0.001$ ) polymeric color than control juice receiving no additive following five weeks of storage. Therefore, a second study was performed with glutathione in combination with lipoic and ascorbic acids (concentration of 500 mg/L each) in an effort to use antioxidant recycling to achieve a synergistic effect. There was no difference in total anthocyanins between glutathione and any of the combinations. However, each glutathione treatment; glutathione, glutathione + lipoic acid, and glutathione + lipoic acid + ascorbic acid had 18.2, 18.8, and 21.9% greater total anthocyanin retention (each  $p<0.0001$ ) following five weeks of storage, respectively than the control. There was a similar protective effect of treatments in percent polymeric color during storage suggesting that antioxidant recycling had no protective effect relative to glutathione alone. There were no notable changes in ellagitannin or flavonol content among additives or over storage. Two flavonols, quercetin-3-glucuronide and quercetin-3-pentosyl-glucuronide, were identified and quantified in blackberries for the first time.

## The Bactericidal Effect of Wild Blueberries on *Listeria Monocytogenes*

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The survival of *Listeria monocytogenes* was evaluated on wild IQF blueberries provided by Jasper Wyman & Son. The exterior of the frozen wild blueberries was artificially inoculated with a five-strain cocktail of *L. monocytogenes* and then stored at refrigeration (4°C) and frozen conditions (-18°C) for up to 28 days. After 3 days of storage, *L. monocytogenes* was not detected in samples of wild blueberries that were stored at refrigeration temperature, however, the population level remained stable under frozen conditions. For three trials conducted, a substantial population level of *L. monocytogenes* (estimated 2.7 to 3.5 logs) died-off immediately after inoculation. This phenomenon was not observed when the study was performed using frozen peas, thus suggesting that the blueberries exerted a bactericidal effect against this foodborne pathogen. Furthermore, as the pH of the blueberries ( $3.48 \pm 0.03$ ) was neutralized within seconds using 100 mM phosphate buffer, the acidic pH of the blueberries does not, alone, explain the rapid and substantial initial die-off of *L. monocytogenes* observed in this study. Other researchers have reported that the low pH, sugars, acids, and salts of blueberries and other berries were not solely responsible for the antimicrobial effects observed. Additional investigation is needed in order to determine the antimicrobial compounds responsible for bacterial inactivation and the mechanism in which these antimicrobial compounds act against *L. monocytogenes*.

# Beneficial Effects of Nordic Lingonberry, Blackcurrant and Bilberry Consumption on Adiposity, Low-grade Inflammation and Gut Microbiota Composition in High-fat Fed C57BL/6J Mice

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**Objective:** The increasing prevalence of obesity is a worldwide health problem, and closely associated with development of metabolic disorders including insulin resistance, nonalcoholic fatty liver disease and type 2 diabetes. Hence, there is a great need to identify dietary strategies for aiding in prevention of obesity and related diseases. We have investigated the potential of different berries to mediate beneficial health effects in a mouse model of diet-induced obesity and prediabetes.

**Methods:** Male C57BL/6J mice were fed a high-fat diet (45 E%) for 13 weeks supplemented with different berries (20%); lingonberry, blackcurrant, bilberry, raspberry, blackberry, açai, crowberry and prunes. Body weight and food intake were monitored continuously, and in the end of the study plasma and organs were collected. Liver tissue was subjected to global gene expression analysis. In a follow-up study, two different batches of lingonberries (Lingon1 and Lingon2, 20%) were evaluated in similar manner with the addition of an analysis of cecal microbiota composition by 16S rRNA sequencing.

**Results & Discussion:** We found that supplementation with lingonberries, blackcurrants and bilberries significantly reduced body weight gain, insulin resistance, low-grade inflammation and hepatic lipid accumulation in C57BL/6J mice fed a high-fat diet. Supplementation with raspberries, crowberries, blackberries or prunes had no or small effects, whereas açai berries promoted development of obesity and fatty liver compared to the control group receiving high-fat diet without berries.

Global hepatic gene expression analysis revealed that the phenotype in the lingonberry and bilberry groups was coupled to an anti-inflammatory effect, including downregulation of acute-phase proteins and inflammatory mediators. Mice receiving açai displayed an upregulation of steatosis markers and genes related to lipid synthesis, in line with the exacerbation of high-fat-induced fatty liver in these mice.

Different batches of lingonberries were found to have different capacity to prevent obesity. In contrast, both batches prevented low-grade inflammation, metabolic endotoxemia and modified the gut microbiota composition in high-fat fed mice.

**Conclusion:** The novel finding of the capacity of lingonberries to counteract negative outcomes of an unhealthy diet should be further evaluated, and may be useful in designing dietary strategies aimed at preventing metabolic disease.

# HPLC-MS/MS Analysis of Anthocyanins and Their Phenolic Acid Metabolites in Human Urine and Plasma Using Simple Sample Preparation Methods

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Anthocyanins are responsible for the blue, purple, and red color of many fruits, flowers, and leaves. Many investigations have associated the intake of anthocyanins with a reduced incidence of diseases such as cardiovascular disease, diabetes mellitus, and cancer. Numerous research articles are dedicated to studies on the absorption, distribution, metabolism and excretion of anthocyanins.

An HPLC-MS/MS method was developed to analyze anthocyanins in urine and plasma. The high performance liquid chromatography (HPLC) method employed a gradient elution system with a Synergi RP-Max column (250 x 4.6 mm, 4 µm) and an API 4000 mass spectrometer. The degradation of anthocyanins was minimized using dilute-and-shoot and protein precipitation sample preparation methods for urine and plasma, respectively. The method has been used to analyze anthocyanin concentrations in urine and plasma samples from volunteers administered saskatoon berries. Cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-xyloside, malvidin-3-galactoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-glucoside, delphinidin-3-rutinoside and quercetin-3-galactoside were identified in plasma and urine samples.

A second HPLC-MS/MS method was developed to analyze phenolic acid metabolites from anthocyanins using the same column and instrumentation. The assay also employs dilute-and-shoot and protein precipitation sample preparation methods for urine and plasma, respectively, which minimize the degradation of anthocyanins. The assay has been used to quantify phenolic acids in plasma and urine samples from volunteers administered saskatoon berries. Plasma and/or urine concentrations of some phenolic acids increase following administration of saskatoon berries. These include protocatechuic acid (PCA), vanillic acid, ferulic acid, hippuric acid, syringic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, caffeic acid, and 2,5-dihydroxybenzoic acid.

## Metabolic Fate of Strawberry Polyphenols after Chronic Supplementation in Healthy Older Adults

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Strawberries are considered as a functional fruit due to the presence of wide array of nutrients including polyphenols such as anthocyanins, procyanidins and ellagitannins. These polyphenols are absorbed and metabolized to various phenolic metabolites/conjugates in the body which may play an active role in disease prevention or protection. In the present study, we explored the metabolic fate of strawberry polyphenols after chronic (90 days) supplementation of freeze-dried strawberry (24 g/d, equivalent to 1 cup of fresh strawberries) in 18 healthy older adults (66.7 ± 4.4 y). Blood samples were collected at baseline (t=0 h) and 2 h post consumption on day 1 (no treatment), day 45 and day 90. A pooled plasma sample (t= 0 and 2 h) from six randomly selected subjects was used to identify the phenolic metabolites/conjugates using ultra high-performance liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometer. Parent anthocyanins and their conjugated metabolites were identified including cyanidin-3-O-glucoside, pelargonidin-3-O-rutinoside, pelargonidin-3-O-glucoside, pelargonidin glucuronide and pelargonidin sulfate. We were able to identify urolithin A glucuronide in the plasma samples for the first time after strawberry consumption. A total of 18 phenolic acid metabolites (glucuronides and sulfates) were identified. Hippuric acid sulfate, vanillic acid glucuronide, isovanillic acid glucuronide, 2, 3-dihydroxybenzoic acid and p-coumaric acid were observed only in 2 h plasma samples compared to 0 h samples. Our results suggest that strawberry polyphenols are absorbed and extensively metabolized resulting in the production of various phenolic acid derivatives and their conjugates, all together contributing to the bioavailability and beneficial effects associated with strawberry consumption. A complete set of quantitative data will be presented at the meeting.

## Investigating Glycaemic Effects Following Flavonoid-Rich Blueberry Drink Consumption: An Acute Dose Response Study in Healthy Young Adults

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Research indicates that flavonoid consumption is associated with benefits for the postprandial glycaemic response, such as an attenuated peak and an extension of the glucose curve. This “blunted” glycaemic response is associated with health benefits such as a reduced risk of type 2 diabetes and benefits to cognitive function. The aim here was to investigate the impact of blueberry anthocyanins on the postprandial glucose response. In a counterbalanced crossover design, seventeen healthy young adults consumed a range of doses of freeze-dried blueberry powder, in smoothie form, in both sugar-matched and no-added-sugar conditions. Plasma glucose was assessed with capillary sampling at baseline and at regular postprandial intervals up to 2.5 hours. Blueberry anthocyanins were observed to significantly extend the postprandial glucose response beyond the period observed for a sugar-matched control, in a dose-dependent manner. In addition, blueberry consumption was associated with a reduce glucose peak, although in incremental area under the curve values were not significantly affected. These findings indicate that blueberry consumption is beneficial for regulation of the glycaemic response. This is of particular relevance to populations with and at risk of type 2 diabetes in whom regular ingestion of blueberry flavonoids may deliver significant metabolic and cognitive benefits.

# Red Raspberry (*Rubus idaeus* L.) Polyphenols: from Food Chemistry to Chemical Metabolites in Human Biological Specimens

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**Background:** Red raspberries contain a variety of polyphenols, characterized by anthocyanins and ellagitannins. Some of the anthocyanins can be absorbed intact in their glycosylated form, while others may pass to the colon where they are degraded to phenolic acids/metabolites by gut microbiota before absorption. Ellagitannins are hydrolysable tannins metabolized by the gut microbiota to produce urolithins. Collectively, the absorbed phenolic compounds (parent compounds, degradant or microbial metabolite molecules) are subject to xenobiotic metabolism in the intestine, liver, and/or kidney, forming methylate, glucuronide, and sulfate conjugated metabolites. Acute and chronic exposure to raspberry polyphenols may alter metabolite patterns depending on adaptations in xenobiotic machinery and/or microbiota composition. Understanding the metabolic fate of these compounds and their composition in different biological specimens relative to exposure will aid in study design development for health benefits, including mechanism of actions studies.

**Objectives:** The present study aimed to characterize red raspberry polyphenols and their metabolites in human biological samples (plasma, urine and breast milk) after acute and chronic intake of red raspberries. **Methods:** Fruit and biological samples from two pilot studies in our lab were analyzed using ultra high performance liquid chromatography (UHPLC)-based methodology coupled with quadrupole time-of-flight (QTOF) and triple quadrupole (QQQ) mass spectrometers. The chromatographic separation of anthocyanin and urolithin derivatives were achieved on a reversed-phase Poroshell C18 stablebond column (2.1×150mm, 2.7µm), while that of the phenolic acids derivatives were achieved on a Pursuit 3 PFP column (2.0×150 mm, 3 µm). Accurate mass, fragmentation patterns, multiple reaction monitoring (MRM) transitions and other important mass spectral characteristics were determined and used to characterize parent polyphenols and their metabolites in biological specimens. The identity of compounds was confirmed using standards when available. One of the studies also characterized the changes of gut microbiome composition after chronic red raspberry intake using 16S rRNA sequencing methodology.

**Results:** The most abundant polyphenols in red raspberries include cyanidin 3-sophoroside, cyanidin 3-glucoside, sanguin H6 and lambertianin C. Acute study results: 2 h post red raspberry consumption, cyanidin 3-sophoroside and cyanidin 3-glucoside were both detected in the plasma and urine samples, while only cyanidin 3-sophoroside was detected in the 2 h post breast milk samples. Chronic study results: after at least one week consistent red raspberry consumption, microbial-derived metabolites, including Urolithin A glucuronide (the main metabolite of sanguin H6 and lambertianin C), hydroxybenzaldehyde, hydroxyphenylacetic acid, dihydroxybenzoic acid were detected in the plasma, urine and breast milk samples. The consistent exposure of red raspberry polyphenols to the gut microbiota changed gut microbiome composition from baseline by increasing Bacteroidetes/Firmicutes ratio. Glucuronide, methylate and sulfate conjugates of anthocyanins and phenolic acids were detected in plasma, urine and breast milk samples although composition differed based on source of biological specimen and raspberry intake.

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# Association Between Plasma Phenolics and Improved Cognition in Blueberry-Supplemented Older Adults

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Research in both human and animals has demonstrated that cognitive function decreases during aging. These functional declines may be caused by long-term increases in and susceptibility to oxidative stress and inflammation. A growing body of research shows that dietary supplementation with blueberries can improve cognition during aging. Phenolic compounds, found in blueberries, are thought to be responsible for this cognitive enhancement due to their antioxidant and anti-inflammatory properties. In a recent clinical study conducted by our laboratory, healthy older adults (ages 60-75 y) were tested in a randomized, double-blind, placebo-controlled trial in which they consumed freeze-dried blueberry (equivalent to 1 cup blueberries) or placebo powder. Participants completed a battery of cognitive tests and provided blood samples at baseline, and again following 45 and 90 days of intervention. Participants in the blueberry group showed enhanced executive function as evidenced by significantly fewer repetition errors in the California Verbal Learning test (CVLT-II;  $p = 0.031$ ,  $p^2 = 0.126$ ) and a reduced switch cost on a task-switching test ( $p = 0.033$ ,  $p^2 = 0.09$ ) across study visits, relative to controls. Levels of anthocyanins and phenolic acids were measured in plasma at fasting and at two hours following breakfast, which included the supplement. Among participants in the blueberry group, change in CVLT-II repetition errors, from baseline to 90 days, was related to changes in fasting levels of hippuric acid and postprandial levels of syringic acid ( $p = 0.002$ ; multiple  $r$ -squared: 0.552). Change in switch errors from baseline to 90 days was predicted by changes in postprandial levels of plasma ferulic acid-glucuronide ( $p = 0.031$ ; multiple  $r$ -squared: 0.258). These findings show that the addition of easily achievable quantities of blueberry to the diets of older adults can improve some aspects of cognition by increasing levels of specific polyphenols.

# Nutraceutical Study of Costa Rican Blackberry (Rubus Sp)

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The content of total polyphenols and antioxidant activity was evaluated in blackberry leaves at two stages of maturation young leaves (YL) and old leaves (OL) and in fruits at three stages of maturation (green, pink and ripe). Samples evaluated were collected from six farms (Trinidad, Luchita, Buena Vista, Dota, and Cedral, Division) in Costa Rica varying in geographical and environmental growing conditions.

It was determined by the Folin-Ciocalteu method that the leaves had higher levels of total phenolics than the fruit over all growing location. The highest content was found in young leaves collected at the farms Luchita, Buena Vista and Division ( $230 \pm 27$ ,  $220 \pm 6$  and  $226 \pm 14$  mg GAE/g DW, respectively), which did not present statistically significant differences between them. The fruit had a higher concentration of total phenolics at the green stage than in the pink and ripe stages, with fruit at the ripe stage having the lowest concentration, this tendency was observed in samples from all the farms studied.

The antioxidant activity determined by the ORAC method was generally higher in leaves than in fruits, with the highest content ( $3322 \pm 10$   $\mu\text{mol TE/g MS}$ ) present in young leaves obtained from the Trinidad farm. However, in some cases no significant differences in ORAC were observed between young and old leaves. The antioxidant activity in fruit was higher in the green fruit than at the two later stages of ripening in samples collected from all locations. When comparing antioxidant activity with total phenolic concentration, a clear trend was observed in which samples with the highest concentration of total phenolics showed the highest antioxidant activity.

The antimicrobial activity of leaf and berry extracts was determined against two gram-positive and two gram-negative bacteria at a concentration of McFarland 0.5. No marked differences in antimicrobial activity were observed between fruits and leaves. In the case of the gram-positive bacteria, the greatest inhibition, 65% was obtained with extracts from green and the pink fruit. The leaf extracts showed greater inhibition of gram negative bacteria than fruit extracts.





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