BERRY HEALTH BENEFITS SYMPOSIUM

The only conference focusing solely on berries & human health.

Symposium Pre-Proceedings

2015 Berry Health Benefits Symposium
October 13-15, 2015 | Madison, WI- USA

The Berry Sessions
Tuesday, October 13th - Ballroom C/D- Level 4

BERRY SESSIONS

8:30 –Berry Session 1
Berryology 101 -
Understand the terminology used in speaking about berry health and a look at the ORAC debate and how to express berry health benefits beyond antioxidants.
Dr. Navindra Seeram

9:30 – BREAK sponsored by Driscoll's ONLY THE FINEST BERRIES™

9:45 – Berry Session 2
Communicating Berry Research to The Dietary Guidelines for Americans Current Research Forum –
A look at how the National Berry Crops Initiative its member groups and others are working with researchers to advance the scientific evidence on berries and health through research into consumer attitudes, buying habits and current research on berries and health.
Dr. Britt Burton-Freeman & Dr. David Stuart

10:45 – Berry Session 3
Translating Science into Consumer Friendly Messaging: A Registered Dietitian's Perspective-RD's will discuss ways to take Scientific studies and create educational and media programs easily understood by everyone.
Frances Largeman-Roth, RDN
Victoria Retelny, RDN, LDN

Noon- Lunch
Community Terrace- Level 2

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Scientific Presentations

Tuesday, October 13th - Ballroom C/D- Level 4

Berries and Heart Health

1:00 – 1:15 –**Current Research Review**
Chair overview by Dr. Britt Burton Freeman, Institute for Food Safety and Health at Illinois Institute of Technology

1:15 – 1:40 – **Berry anthocyanins and cardiometabolic health**
Dr. Aedin Cassidy, University of East Anglia

1:40 – 2:05 – **Berries and Lipids in Cardiovascular Disease: observations and recommendations**
Dr. Arpita Basu, Oklahoma State University

2:05 – 2:30 – **Wild Blueberries Attenuate Risk Factors of the Metabolic Syndrome**
Dr. Dorothy Klimis-Zacas, University of Maine

2:30 – 2:55 – **Berry consumption and cardiometabolic disease**
Dr. Indika Edirisinghe, Institute for Food Safety and Health (IFSH)

3:00- BREAK sponsored by Driscoll's ONLY THE FINEST BERRIES

Berries and Gut Health/Gut Microflora

3:15 – 3:30 –**Current Research Review**
Chair Overview by Dr. Jess Reed, University of Wisconsin-Madison

3:30 – 3:55 – **Microbial- and mammalian-mediated metabolism of dietary flavonoids in the gastrointestinal tract: impact on bioavailability**
Dr. Alan Crozier, University of California, Davis, CA

3:55 – 4:20 – **Interactions between gut microbes and dietary anthocyanins**
Dr. Federico Rey, University of Wisconsin

4:20 – 4:45 –**Berries to fight the metabolic syndrome: what are the mechanisms?**
Dr. Andre Marette, Laval University

4:45 – 5:10 – **The role of microbes in the relationship between berries and human health**
Dr. Simone Gugliemetti, University of Milan

7:00- Welcome Dinner sponsored by Ocean Spray, INC.

Community Terrace

Level 2

Ocean Spray

Scientific Presentations
Wednesday, October 14th – Ballroom C/D- Level 4

Berries and Brain Aging

8:00 – 8:15 – **Current Research Review**
Chair overview by Dr. Barbara Shukitt-Hale, USDA/Tufts University

8:15 – 8:40 – **Berry supplementation to mitigate neurocognitive decline**
Dr. Robert Krikorian, University of Cincinnati

8:40 – 9:05 – **The effect of blueberry consumption on cognitive abilities in 65- to 79-year-olds: a 6-month randomized controlled trial.**
Dr. Carol Cheatham, University of North Carolina

9:05 – 9:30 –**Effects of berry supplementation on mobility and cognition among older adults**
Dr. Marshall Miller, Tufts University

9:30 – 9:55 – **Effects Of Flavonoid-Rich Blueberry Interventions On Cognitive Behaviour In 7-10 Year Old Children**
Dr. Claire Williams, University of Reading

10:00 – 10:15 AM BREAK sponsored by Driscoll's ONLY THE FINEST BERRIES™

Berries and Cancer

10:15 – 10:30 – **Current Research Review**
Chair overview by Ramesh Gupta, University of Louisville

10:30 – 10:55 – **Crops to the Clinic" Cancer Prevention Research with Berries**
Dr. Steve Clinton, Ohio State University

10:55 – 11:20 –**Cancer prevention and therapeutic efficacy of berry bioactives**
Dr. Farrukh Aqil, University of Louisville

11:20 – 11:45 – **Black Raspberries in Clinical Studies: Past, Present and Future**
Laura Kresty, Medical College of Wisconsin

11:45 – 12:10 – **Bilberry in IBD – just getting blue or getting better?**
Luc Biedermann, University Hospital of Zurich

12:15 PM – 1:00 PM – LUNCH- Sponsored by:
Community Terrace Level 2

FSGA Florida Strawberry Growers Association

Scientific Presentations
Wednesday, October 14th – Ballroom C/D- Level 4 (CONT.)

Berry Compositional Chemistry and Biological Effects

1:00 – 1:15 – **Current Research Review**
Chair overview by Dr. Navindra Seeram, University of Rhode Island

1:15 – 1:40 – **The Modernized Analytic Toolbox for Authentication, Standardization and Efficacy Evaluation of Natural Products**
Dr. Chris Kruger, University of Wisconsin-Madison

1:40 – 2:05 – **The effects of strawberry bioactive compounds on human health: a possible clue on the molecular mechanisms involved in the prevention of different chronic diseases**
Dr. Maurizio Battino, Università Politecnica delle Marche

2:05 – 2:30 – **Bioavailability and bioactivity of phytochemicals in cranberries**
Dr. Liwei Gu, University of Florida

2:30 – 2:55 – **Digging deeper into the anti-infective chemistry of cranberry**
Dr. David Rowley, University of Rhode Island

3:00-3:15 PM – BREAK sponsored by Cott

Berries and Metabolism

3:15 – 3:30 – **Current Research Review**
Chair Overview by Dr. Ron Prior, University of Arkansas

3:30 – 3:55 –**Newly discovered bioactive metabolites of berry anthocyanins: potential health effects and implications for future research**
Dr. Colin Kay, University of East Anglia

3:55 – 4:20 – **Berries and cardiovascular health: is it really the anthocyanins**
Dr. Ana Rodriguez Mateos, University of Reading

4:20 – 4:45 – **Raspberry ellagitannin metabolites in experimental models of chronic disease**
Dr. Daniele Del Rio, Univerity of Parma

4:45 – 5:10 – **Aronia an up and coming healthy berry**
Dr. Emilie Fromentin, NATUREX-DBS

Wednesday, October 14th – *Keynote Dinner- 7:00 PM*
Community Terrace Level 2

Our Keynote Speaker will be Dr. Amy Howell, Associate Research Scientist at the Marucci Blueberry and Cranberry Research and Extension Center of Rutgers University.

An outstanding presentation relating further research and new knowledge on berries and health. Dr. Howell is known for her groundbreaking work on cranberry proanthocyanidins (PAC's).

Keynote Speaker- Dr. Amy Howell

"Cranberry – To the Bladder and Beyond!"

Thursday, October 15th

The Wisconsin Cranberry Discovery Tour: Cranberry Harvest Tour and Closing Dinner

Enjoy the beauty of fall in Wisconsin and experience the excitement of cranberry harvesting while visiting two local farms, one harvesting for fresh market and the second for the processed market. A visit to other cranberry processing and history sites will be followed by a closing reception and dinner. This full day tour will include bus transportation, lunch and dinner.

Sponsored By:

WISCONSIN STATE CRANBERRY GROWERS Association

Bus will pick us up at the main entrance of the Madison Concourse Hotel

Departure time: 9:00 AM
Return time: 9:00 PM

Please plan on meeting in main lobby at 8:30am so we can depart on time.

We will be visiting:

- ***Cutler Cranberry Farms (lunch provided)***
- ***Habelman Brother Cranberry Farm***
- ***Ocean Spray Receiving Center***

Dinner at Paul Bunyan's Restaurant sponsored by naturipe

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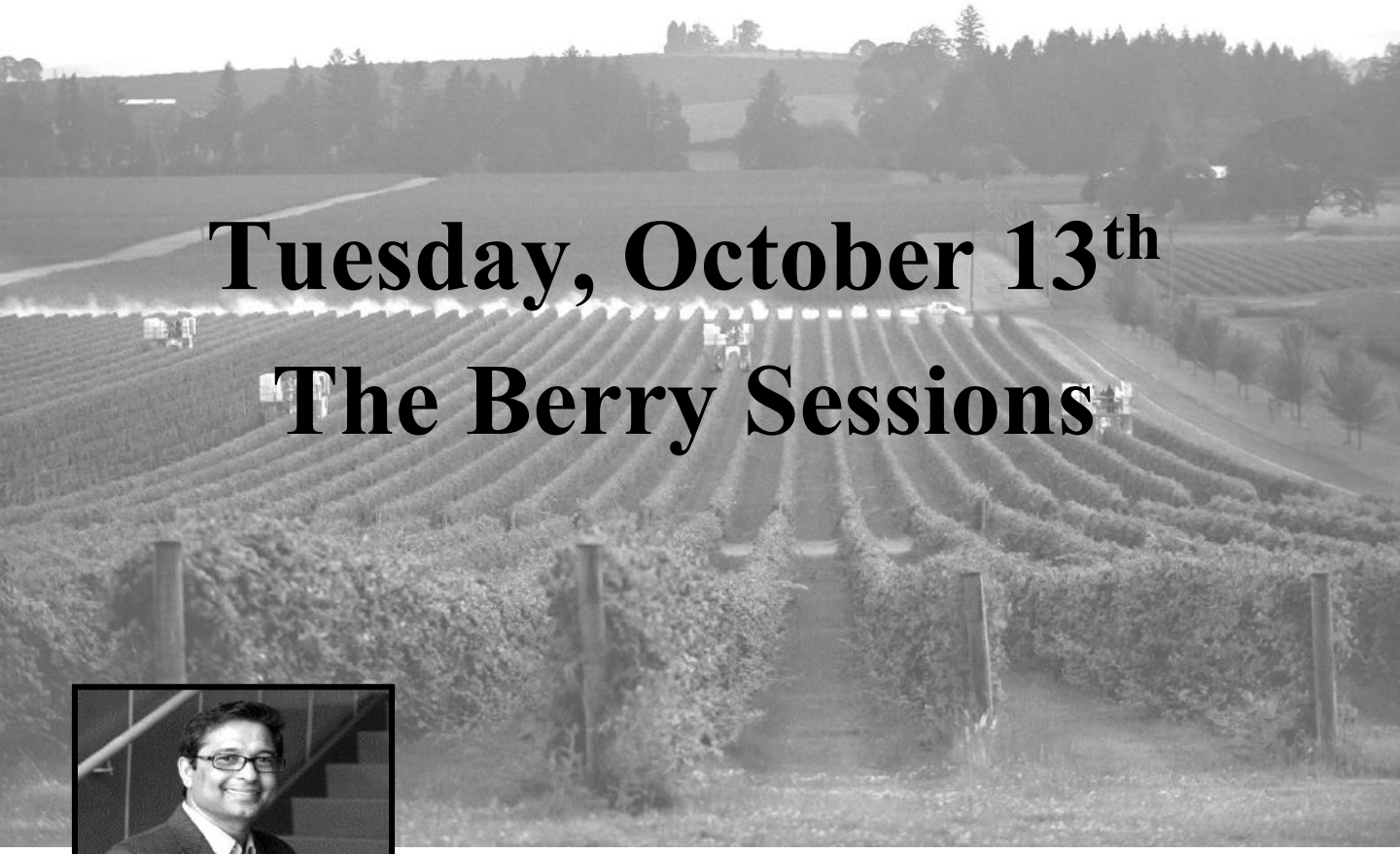


Tuesday, October 13th
The Berry Sessions

Navindra Seeram Ph. D.

University of Rhode Island

Berryology 101

Navindra P. Seeram, Ph.D., 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 Assistant Adjunct Professor in the UCLA School of Medicine.

His research group, the Bioactive Botanical Research Laboratory, investigates plant foods and natural products for preventive and therapeutic effects against chronic human diseases. Dr. Seeram has co-authored over 121 original peer-reviewed research articles, 7 review articles, 16 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 American Chemical Society's Division of Agricultural and Food Chemistry Division 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 based on Web of Science indexed citations from 2002-2012) 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.

Britt Burton Freeman, Ph.D.

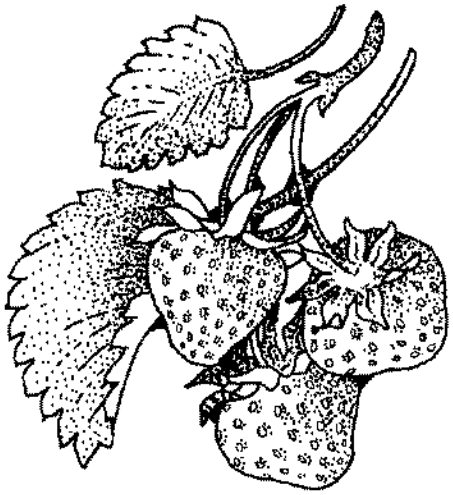
Institute for Food Safety and Health at Illinois Institute of Technology

Communicating Berry Research to The Dietary Guidelines for Americans Current Research Forum

Britt Burton-Freeman, Ph.D. is the Director of Nutrition and Health Promoting Foods platform leader at the National Center for Food Safety and Technology (NCFST), Illinois Institute of Technology. Dr. Freeman has been involved in obesity and metabolic disease research for over 15 years, including basic science and clinical research in academic, biotechnology and drug development settings.

Dr. Freeman's current research interests are in mitigating disease process through dietary approaches focused on the health promoting properties of whole foods. Specific disease targets are vascular disease and obesity, including food intake regulation. In her current appointment, she leads a public health initiative with FDA/CFSAN to develop and provide underpinning science for comprehensive approaches using innovative processing solutions to support the availability of safe food with health opportunities.

Dr. Freeman is an active member of multiple professional societies dedicated to health and disease abatement including the American Society for Nutrition, the Obesity Society and the Society for the Study of Ingestive Behavior. Dr. Freeman earned a M.S. and Ph.D. in Nutrition Science with an emphasis in Endocrinology and Physiological Chemistry at the University of California, Davis.



DAVID A. STUART, Ph.D

Food & Nutrient Impact, LLC
University of California, Berkeley

Communicating Berry Research to The Dietary Guidelines for Americans Current Research Forum

BIOGRAPHY: DAVID A. STUART, Ph.D.
David A. Stuart Ph.D. is Founder and Principal of "Food & Nutrient Impact, LLC", a located in Hershey PA focusing on research and advice on healthy foods, on cacao and on agricultural sustainability.

He holds a B.S. in Biology and minor in Chemistry from California State University, Sacramento. He earned his M.A. and Ph.D. in Botany and Plant Physiology from the University of California, Berkeley where he studied the biophysics of plant cell growth.

David earned an Executive Program degree in Agribusiness from the Smeal School of Business at The Pennsylvania State University in 1999.David worked for Plant Genetics, Inc. in Davis CA for eight years where he was leader of their cell biology, plant pathology and the genetic improvement programs. Work there was done in association with some familiar companies such as Kirin Breweries, McCormick Spices and Best Foods (now part of Unilever).

David began work at The Hershey Company in 1989 where he was a manager or director the Research and Development Department. His career there literally spanned ***Cocoa Bean to Chocolate Bar.*** He began as Manager of Biochemistry where he immediately became involved with cocoa, its genetics, biochemistry and field production. He was active as a leader in the Chocolate Industry's Cocoa Biotechnology programs which have led to advances in variety cloning, new variety development and the Cocoa Genome which was published in 2010. Eight years ago, he developed the proposal leading to the Hershey Center for Health and Nutrition becoming Center Director. He led a group of nine Ph.D. scientists responsible for the characterization of the flavanol chemistry of cocoa, flavanols levels of market basket surveys, flavanol loss during processing, shelf stability of flavanols, clinical trials using commercially available dark chocolate and cocoa, product labeling and web-site development. He worked with national and international groups to set the standard testing protocols for these naturally occurring bioactive compounds. His area was also responsible for the development of new cocoa and high-flavanol ingredients for use in product development as well as studies on the absorption of flavanols into the blood stream and on the impact of these compounds on human metabolism. He was recently awarded the life-time achievement award by the National Confectioners Association for his contributions to the industry.

David has authored more than 35 publications, an inventor on eight patents, including 17 papers and published presentations since the Center was formed. He has also participated in numerous lectures at universities, presentations at meetings and at scientific conferences.
Dave Berry Meeting Bio m5 d31 y13

Translating Science into Consumer Friendly Messaging: A Registered Dietician's Perspective

Frances Largeman-Roth, RDN

Author and Health Expert

Frances Largeman-Roth, RDN, is a *New York Times* best selling author and nationally recognized health expert. Frances was the Food and Nutrition Director at *Health* magazine for nearly eight years. She writes for *Parents, BabyCenter, Today.com, Cooking Light* magazine and other publications. Frances is a sought after spokesperson and provides private nutrition counseling to clients through her HealthyHousecalls.

Frances is a frequent guest on national TV, including the Today Show, Good Morning America, Access Hollywood Live, CNN, The Rachael Ray Show, and The Dr. Oz Show. She has also lent her expertise as a judge for Food Network Challenge and The James Beard Awards. Frances is a contributor and on-air spokesperson for *Cooking Light* magazine.

Frances is the author of *Feed the Belly: The Pregnant Mom's Healthy Eating Guide* and co-author of the bestselling *The CarbLovers Diet* and *The CarbLovers Diet Cookbook.* Her latest cookbook is *Eating In Color: Delicious, Healthy Recipes for You and Your Family*. Frances earned her undergraduate degree from Cornell University and completed her dietetic internship at Columbia University.

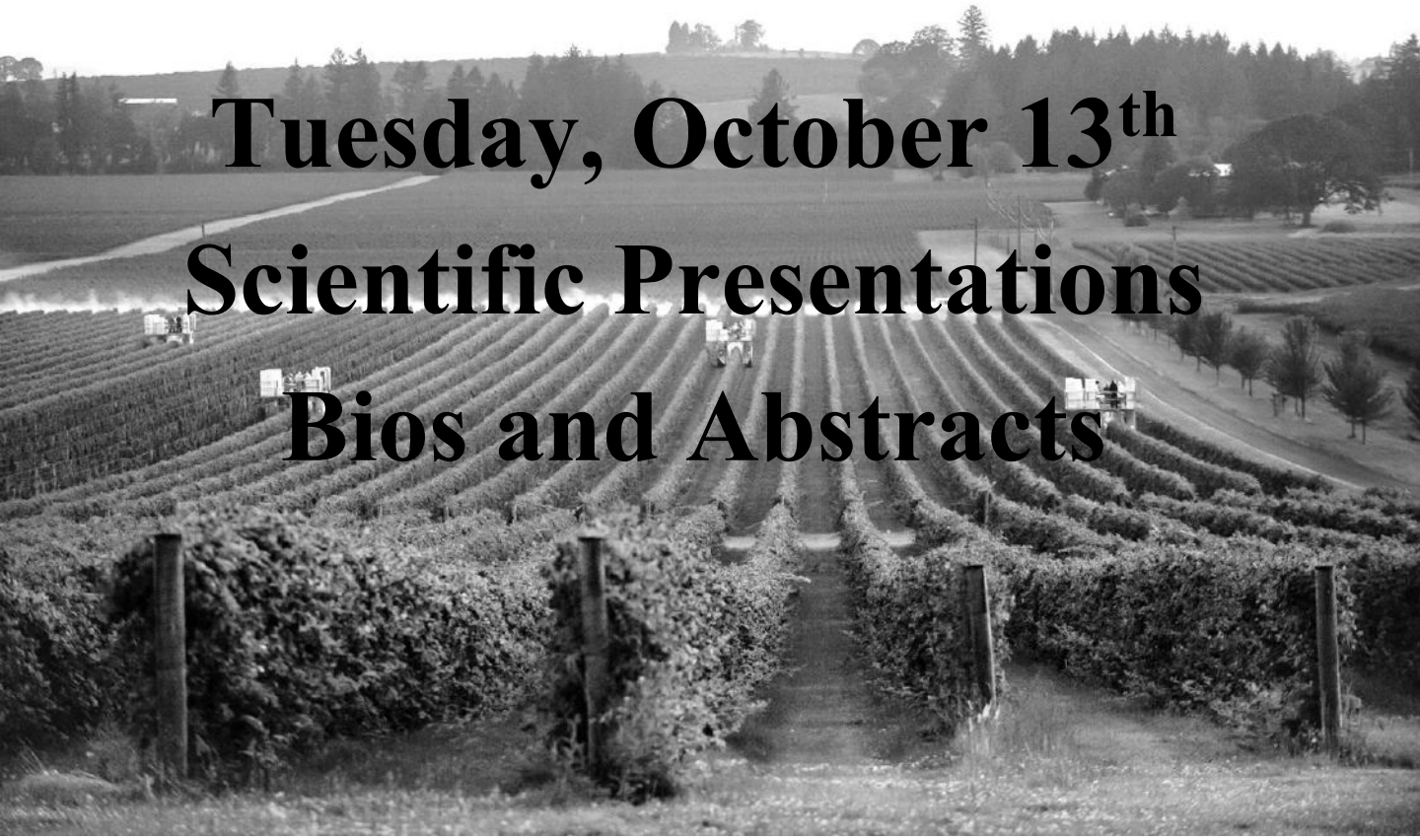
•Frances lives in Brooklyn, NY, with her husband and three kids. To learn more, go to www.franceslargemanroth.com, or follow her on social media @FrancesLRothRD.

Victoria Retelny, RDN, LDN

Health Expert, Culinary Media Consultant

Vicki Shanta Retelny, RDN, LDN, is a nationally-recognized lifestyle nutrition expert, culinary and media consultant. Her book *The Essential Guide to Healthy Healing Foods* is an empowering evidence-based exploration into the landscape of food, which encourages readers to evolve their eating for improved health, happiness and longevity. Her new book, *Total Body Diet For Dummies* is due for release later this year. As a mother of two, her passion for translating nutrition science into usable, real-life messages combined with her culinary capabilities educates consumers on appreciating healthful ingredients and delicious flavors while creating tasty, nutritious meals. With a particular interest in mindful eating, her byline has appeared in dozens of national publications, including *The Costco Connection, EatingWell Magazine* and *Chicago Health Magazine*. Vicki has appeared on *ABC World News Tonight*, contributes to *WGN-TV's Medical Watch*, *CBS-TV* and *ABC-TV* in Chicago. She lives to eat well with her husband, two active youngsters and their precocious pet pug.

Vicki's blogs her recipes and writings at SimpleCravingsRealFood.com.



Tuesday, October 13th
Scientific Presentations
Bios and Abstracts

Berries & Heart Health

Britt Burton Freeman, Ph. D.

Session Chair
Institute for Food Safety and Health at Illinois Institute of Technology
Current Research Review

Dr. Freeman's current research interests are in mitigating disease process through dietary approaches focused on the health promoting properties of whole foods. Specific disease targets are vascular disease and obesity, including food intake regulation. In her current appointment, she leads a public health initiative with FDA/CFSAN to develop and provide underpinning science for comprehensive approaches using innovative processing solutions to support the availability of safe food with health opportunities.

Dr. Freeman is an active member of multiple professional societies dedicated to health and disease abatement including the American Society for Nutrition, the Obesity Society and the Society for the Study of Ingestive Behavior. Dr. Freeman earned a M.S. and Ph.D. in Nutrition Science with an emphasis in Endocrinology and Physiological Chemistry at the University of California, Davis.

Dr. Aedin Cassidy, Ph.D.

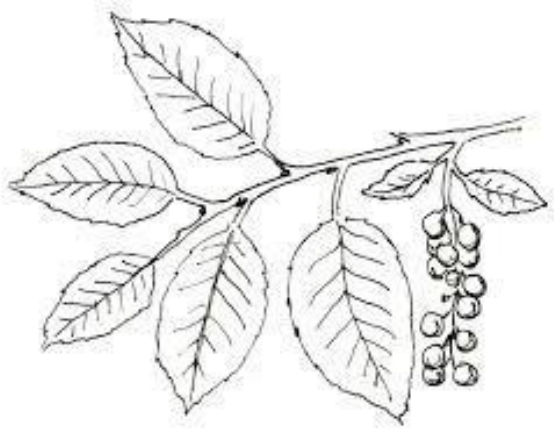
University of East Anglia

Berry Anthocyanins and Cardiometabolic Health

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.



Berry Anthocyanins and Cardiometabolic Health

Our understanding of the metabolism and health effects of anthocyanins has increased significantly recently. Data from prospective cohort studies highlight the beneficial impact of habitual intakes of anthocyanins on both biomarkers of cardiovascular (CV) risk and disease outcomes including myocardial infarction (MI) and type 2 diabetes (1-4). We showed that a higher intake of anthocyanins was associated with a 32% reduction in risk of MI and this inverse association was independent of established dietary and non-dietary CVD risk factors (1). In relation to T2DM, a 15% reduction in risk was observed comparing highest to lowest quintiles of anthocyanin intake (2). Currently there are limited validated biomarkers to integrate intake with the extensive metabolism these compounds undergo in vivo, so our current knowledge is based on dietary intake.

We recently established that anthocyanins are absorbed and metabolised to a greater extent than had been previously reported; 12% of the ingested dose of 13C-labelled anthocyanin was recovered in urine (5,6). However there is wide-inter individual variability in metabolism and the impact of this variability in metabolism on health is distinctly lacking. Nutritionally relevant levels of these downstream phenolic metabolites appear to be more bioactive than the parent molecules with ongoing mechanistic studies suggest that colonic metabolites exert greater vascular and anti-inflammatory activity than the metabolites formed and absorbed in the small intestine (6-8), providing further evidence that the bioactivity of anthocyanins is highly likely attributed to their phenolic metabolites. The large heterogeneity in the bioactivity and bioavailability of anthocyanin metabolites formed following ingestion, including the extensive range of gut metabolites identified, supports a strong-interplay between anthocyanins and the microbiome. Although it is likely that anthocyanin intake alters the composition and function of the gut microbiome and conversely, microflora enhances the metabolism of anthocyanins, this bidirectional relationship has never been addressed in flavonoid research and the gut microbiota may provide the missing link in explaining the health effects.

To date, very few RCTs have examined the impact of anthocyanins on cardio-metabolic health relative to other sub-classes such as the flavan-3-ols present in tea and cocoa (9). In several short-term interventions (< 2 month duration), anthocyanin rich food intake resulted in a reduction in both systolic and diastolic blood pressure and favourable changes in arterial stiffness, inflammation and LDL cholesterol levels (10-13). These preliminary RCT data are supported by recent evidence to suggest that inflammation may be a key pathway to explaining beneficial effects of anthocyanins on health (14), and mechanistic studies in cell and animal models are suggestive that an impact on whole body insulin action and vascular function may mediate the observed reduction in disease risk. Therefore, although promising data is emerging from cohort studies, and from cell and animal studies, proof of efficacy from longer-term human RCTs and an understanding of the importance of metabolism (and the microbiome) in relation to clinical efficacy are distinctly lacking.

Key words: anthocyanins, cardiovascular, diabetes, metabolism, microbiome

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Arpita Basu, Ph.D.

Oklahoma State University

Berries and Lipids in Cardiovascular Disease: observations and recommendations

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.

Berries and Lipids in Cardiovascular Disease: observations and recommendations

Nutritional epidemiology provides accumulating evidence on the inverse associations between dietary intakes of polyphenolic flavonoids and CVD. Among the popular sources of dietary flavonoids, berries have gained considerable attention because of their inverse associations with CVD events (1) and CVD risk factors, such as elevated C-reactive protein (CRP) (2), hypertension (3), and type 2 diabetes (4), demonstrated in large prospective studies. These associations have been mostly attributed to the consumption of blueberries, cranberries and strawberries, which are important sources of polyphenols in the U.S. diet (5, 6). Reducing elevated LDL-cholesterol is a key public health challenge. There is substantial evidence from randomized controlled trials (RCT) that a number of foods and food components can significantly reduce LDL-cholesterol (7). Dietary berries deserve special attention on their role in improving serum lipid profiles in adults with CVD risk factors.

Cranberries and strawberries have been associated with lowering blood total and LDL cholesterol in short-term intervention studies (3-12 weeks) in adults with diabetes and CVD. Our group has previously reported the role of dietary strawberries (freeze dried powder) in causing a significant reduction in total and LDL cholesterol, as well as nuclear magnetic resonance (NMR)-derived small LDL particle concentrations in adults with elevated lipid profiles (8). Similar observations have also been reported using dietary strawberries in overweight adults (9) and those with type 2 diabetes (T2D) (10). Studies in obese men have shown the role of low calorie cranberry juice in increasing HDL cholesterol, suggesting a cardioprotective role in these at risk adults (11). In our study in obese adults with the metabolic syndrome, though low calorie cranberry juice did not affect blood glucose and lipids, we noticed a significant decrease in biomarkers of lipid oxidation following cranberry intervention (12). Cranberry extracts have also been shown to decrease total and LDL cholesterol in in adults with T2D (13). Mechanistic studies provide insight into these observations as being caused by the inhibitory actions of berry bioactive compounds, especially the polyphenols in digestive enzyme functions, cholesterol absorption and synthesis (14). Thus, dietary berries hold promise in the management of blood lipids as primary risk factors for CVD. Future clinical trials must focus on dose-response effects of berries on lipid profiles, as well as examine their effects on detailed biomarkers of lipid metabolism, such as apolipoproteins and oxidized lipids in presence of risk factors and advanced forms of CVD.

Key words: Cranberries, Strawberries, LDL cholesterol, HDL cholesterol, Diabetes, Metabolic Syndrome

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Dr. Dorthy Klimas-Zacas, Ph.D.

University of Maine

Wild Blueberries Attenuate Risk Factors of the Metabolic Syndrome

Dorothy Klimis-Zacas, PhD is Professor of Clinical Nutrition and cooperating Graduate Faculty, School of Biomedical Sciences at the University of Maine. She is also cooperating professor of Nutrition and Dietetics at Harokopio University, Athens, Greece and at the Department of Food, Environmental and Nutritional Sciences at the University of Milan, Italy. She received her MS in Human Physiology and PhD in Nutrition Science from the Pennsylvania State University and did post-doctoral training at the Department of Physiological Chemistry, University of Cologne, Germany and the University of Cologne Hospital.

Dr. Klimis-Zacas has been involved in biomedical research exploring the role of trace minerals and dietary bioactives on chronic diseases such as Obesity, Cardiovascular Disease and the Metabolic Syndrome including basic and clinical investigations. Her applied investigations involve cross-cultural studies that utilize dietary interventions to reduce cardiovascular risk in populations both in the United States and the Mediterranean region.
Her past studies have documented the beneficial role of wild blueberries on vascular function, structure and metabolism in normotensive and hypertensive states. Her recent investigations examine the role of wild blueberries on attenuating co-morbidities associated with the Metabolic Syndrome such as Obesity-induced inflammation, dyslipidemia and insulin resistance as well as gene expression related to the above.
Dr. klimis-Zacas was awarded a senior Fulbright Fellowship to the Hellenic School of Public Health, Athens, Greece where she was involved in the Pan European Project EPIC (European Perspective study Into Cancer) and received two Fulbright Specialist awards to the University of Milan, Department of Food, Environmental and Nutritional Sciences. Additionally, Dr. klimis-Zacas was the recipient of the prestigious Fondazione Cariplo Fellowship to lead research on the use of biosensors in exploring dietary approaches for degenerative disease prevention at the University of Milan.

Dr. Klimis-Zacas is the editor of "Manganese in Health and Disease", 'Nutritional Concerns for Women", and has acted as editor-in-chief of "Annual Editions in Nutrition" and member of several editorial boards including the Journal of Nutritional Biochemistry and Council member of the International Society of Trace Mineral Research in Humans (ISTERH). Dr. Klimis-Zacas is a member of many professional societies dedicated to promoting health and preventing disease including The American Society for Nutrition, The International Atherosclerosis Society, The American Academy of Nutrition and Dietetics, The Italian Society of Nutrition, The Hellenic Dietetic Association and many others.

Wild Blueberries Attenuate Risk Factors of the Metabolic Syndrome

The metabolic syndrome (MetS) is a cluster of strictly interrelated risk factors dramatically increasing the risk of developing type II diabetes and cardiovascular disease, which are leading causes of death in the US and worldwide (1). While the diagnostic criteria for MetS involve blood lipid profile, fasting blood glucose, blood pressure, and waist circumference measurements, the development of endothelial dysfunction and a chronic pro-inflammatory, pro-oxidative and pro-thrombotic environment are landmark characteristics of this condition (2). A pro-inflammatory state strongly correlates with oxidative stress, endothelial dysfunction, atherosclerosis and insulin resistance, leading to the hypothesis that inflammation could in fact be the underlying factor linking all the different abnormalities of the MetS (3). Endothelial dysfunction related to a pro-inflammatory state is an imbalance between vasoconstrictor and vasodilator responses resulting in impaired vascular tone, peripheral vascular resistance and organ perfusion, and is one of the earliest events in the development of atherosclerotic lesions (4). The obese Zucker rat (OZR) represents a valid experimental model for the human MetS (5). Due to its genetic profile, it develops between 8 and 20 weeks of age a multitude of detectable abnormalities, including obesity, hypertriglyceridemia and hypercholesterolemia, insulin resistance and hyperinsulinemia, as well as a moderate form of hypertension (6).

Recent evidence indicates that diet and dietary bioactive compounds can play a fundamental role in preventing and reversing endothelial dysfunction and inflammation (7). Both in vitro and in vivo, dietary bioactive compounds such as polyphenols have been shown to affect the expression of genes involved in inflammation and lipid metabolism and modulate the transcriptional activities of different nuclear receptors that control such pathways (8). Wild blueberries (WB) are one of the richest fruit sources of anthocyanins and other polyphenols. Our past studies, have documented the cardioprotective effect of wild blueberries on vascular function in normotensive rats (9, 10) and in spontaneously hypertensive rats (SHR) (11). Furthermore, wild blueberry-enriched diets have been shown to remodel the structure of the aortic extracellular matrix by altering the concentration and sulfation patterns of glycosaminoglycans, both in Sprague-Dawley rats (12) and SHR (13), which may favorably affect signal transduction pathways involved in endothelial function.
Hence, this presentation will document the ability of a wild blueberry-enriched diet to improve risk factors related to the pathogenesis of the MetS such as endothelial dysfunction and chronic inflammation in the Obese Zucker Rat (OZR), a model of the Metabolic Syndrome.
In one set of experiments, thirty-six OZRs and 36 lean controls (LZR) were placed either on a Wild Blueberry-enriched (WB) or a control(C) diet for 8 weeks. Phenylephrine (Phe)-mediated vasoconstriction and acetylcholine (Ach)-mediated vasorelaxation in the aortic vessel were investigated, as well as the contribution of the nitric oxide synthase (NOS) and cyclooxygenase (COX) pathways in each of the above responses by using specific inhibitors. Obese Zucker rats exhibited a reduced vasocontstrictor response to Phe and an exaggerated vasorelaxant response to Ach. The WB diet partially restored Phe-induced constrictor responses and attenuated Ach-induced relaxant responses in OZR. Plasma nitric oxide was significantly attenuated (22.1±1.1 μmol/L, WB vs 25.6±1.4 μmol/L, C, $p \leq 0.05$) with the WB diet. Thromboxane A2 levels in the aortic effluent were not significantly affected in the WB diet group, while PGI2 concentration significantly increased (766.5±92.2 pg/mg aorta in the WB vs 571.7±37.8 pg/g aorta in the C group, $p \leq 0.05$). Downregulation of iNOS and COX-2 expression in the OZR aorta was observed in the WB diet group. In conclusion, WB consumption altered the biomechanical properties of the OZR aorta by partially restoring the impaired Phe-induced constrictor responses, and attenuating the exaggerated response to Ach-induced vasorelaxation.

In another set of experiments, the ability of a wild-blueberry-enriched diet to improve the pro-inflammatory status associated with MetS in the OZR was studied. Circulating levels of key inflammatory markers and their expression in the liver and abdominal adipose tissue were examined in OZR and its genetic control, the lean Zucker rat (LZR), after feeding a control or an 8% WB diet for 8 weeks from age 8 to 16 weeks.
In the OZR, WB consumption resulted in decreased plasma concentrations of tumor necrosis factor (TNF)-α (−25.6%, $P<0.05$), interleukin (IL)-6 (−14.9%, $P<0.05$) and C-reactive protein (CRP) (−13.1%, $P<0.05$) and increased adiponectin concentration (+21.8%, $P<0.05$). Furthermore, expression of IL-6, TNF-α and nuclear factor (NF)-kB was down-regulated in both the liver (−65%, −59% and −25%, respectively) and the abdominal adipose tissue (−64%, −52% and −65%),while CRP expression was down-regulated only in the liver (−25%). In the abdominal adipose tissue, similar trends were also observed in LZR following WB treatment, with decreased liver expression of NF-kB, CRP, IL-6 and TNF-α (−24%, −16%, −21% and −50%) and increased adiponectin expression (+25%). Hence, wild blueberry consumption exerts an overall anti-inflammatory effect in the OZR, a model of the metabolic syndrome.
Overall, regular consumption of dietary-achievable amounts of wild blueberries rich in polyphenols may have a significant impact on improving vascular function and attenuating the inflammatory status associated with the MetS.

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Dr. Indika Edirisinghe Ph.D.

Institute for Food Safety and Health (IFSH)

Berry Consumption and Cardio-Metabolic Disease

Indika Edirisinghe, Ph.D., Assistant Professor of Food Science and Nutrition at the Institute for Food Safety and Health (IFSH), a research consortium of the United states Food and Drug Administration (FDA) and Illinois Institute of Technology (IIT). He is also the associate Director/ Center for Nutrition Research at IFSH. Dr. Edirisinghe has nearly 15 years experiences in the area of nutritional science, biochemistry and molecular biology. His research focuses on the effect of polyphenolic compound on endothelial function, blood pressure regulation, insulin resistance, inflammatory and oxidative stress responses during acute and chronic interventions. The research approach includes human cell culture, animal models and human clinical trials. He has authored over 40 peer reviewed-original research articles and has over 100 total publications including other articles, meeting presentation and book chapters. He is a member of a variety of professional organizations including: The American Society for Nutrition (ASN), The Institute of Food Technologists (IFT) and The American physiological society (APS).

Berry Consumption and Cardio-Metabolic Disease

The increase in cardio-metabolic diseases exerts a great burden on people, healthcare organizations, and society in general and can present sub-clinically long before becoming clinically apparent. Risk factors associated with cardio-metabolic diseases are important targets for control, since most are sensitive to lifestyle modification. Diet is one of the key lifestyle factors involved in the genesis, prevention, and control of cardio-metabolic diseases. Berry fruit containing polyphenolic compounds have been shown to have an encouraging potential to help prevent cardio-metabolic diseases.

This presentation will discuss the findings of recent pre-clinical and clinical research data from our program and other studies that involve human berry fruit supplementation in the context of reducing risk factors associated with cardio-metabolic diseases. The research evidence demonstrates that polyphenolic compounds derived from berry fruits exert several health benefits through direct interactions with cellular receptors or enzymes involved in signal transduction, which may result in modification of several risk factors and biomarkers associated with cardiometabolic diseases. In particular, insulin signaling and glucose homeostasis have been shown to be modulated via mechanisms related to antioxidant and anti-inflammatory pathways in response to berry consumption. Overall, the data from human clinical studies, epidemiology and the considerable basic science evidence comprise an emerging body of science suggesting that berry fruit inclusion in the diet can reduce the risk of biomarkers associated with cardio-metabolic diseases. However, continued investigation of the unique bioactive components, their bioavailability and kinetic metabolic profiles, biological effects, intervention methodologies, and molecular mechanisms are required to establish effective interventions.

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Berries and Gut Health/Gut Microflora

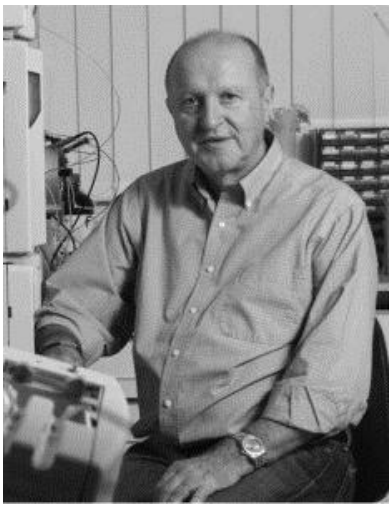
Jess Reed, Ph. D.

Session Chair
University of Wisconsin-Madison

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 25 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 phyochemistry 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 90 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.



Alan Crozier, Ph.D.

University of California, Davis, CA

Microbial- and mammalian-mediated metabolism of dietary flavonoids in the gastrointestinal tract: impact on bioavailability

Alan Crozier's research has focused on the occurrence of dietary flavonoids in berries, fruit, vegetables and beverages including teas and wines and the fate of these compounds within the body following ingestion as they proceed down the gastrointestinal tract and are absorbed and metabolized in both the small and large intestine. He has published over 300 papers, edited several books and is a 2014 Thomson-Reuters Highly Cited Researcher.

Microbial- and mammalian-mediated metabolism of dietary (poly)phenols in the gastrointestinal tract: impact on bioavailability

Berries are a rich source of polyphenolic compounds, including large molecules called procyanidins and ellagitannins. Following consumption of berries these compounds are not initially absorbed as they pass along the gastrointestinal tract. However, when they reach the large intestine the colonic bacteria breakdown procyanidins and ellagitannins to smaller molecules which are absorbed into the bloodstream and there is growing evidence that these colon-derived products play a role in the beneficial effects of berry consumption on human health

Federico Rey, Ph.D.

University of Wisconisin

Interactions between gut microbes and dietary anthocyanins

Education

2006 Ph.D. Microbiology. University of Iowa, Iowa City, IA.
Ph.D. advisor: Dr. Caroline S. Harwood
1998 Licenciado en Bioquimica (equivalent to B.S. and M.S. in Clinical Chemistry). Universidad Nacional de Cordoba, Cordoba, Argentina.

Patents

Rey FE, Harwood CS, Flickinger MC. A structured material for the production of hydrogen. US Patent 7,745,023
Rey FE, Harwood CS. Hydrogen Production from microbial strains. US Patent 20,120,220,006
Gordon JI, Faith JJ, McNulty N, Rey FE, Goodman AL, Kallstrom G, Ridaura V. Cultured Collection of Gut Microbial Community. WO Patent 2,012,122,522

Professional activities

Ad Hoc Reviewer
Journals: Nature Chem Biol, mBio, Journal of the International Society for Microbial Ecology (ISME), Applied Environmental Microbiology, Journal of Biological Chemistry, Journal of Molecular Biology, Scientific Reports, Proceedings B, FEMS Microbiology Reviews.
Funding agencies: NIH (NIBIB, NCCAM), The Canada Foundation for Innovation, Research Foundation – Flanders (FWO)

Interactions between gut microbes and dietary anthocyanins

Anthocyanins are widely distributed in fruits, vegetables and pigmented cereals. Frequent consumption of foods rich in anthocyanins is associated with reduced risk of metabolic and cardiovascular disease (CVD) and food intervention studies have shown that dietary supplementation with fruits rich in anthocyanins can improve clinical and biomedical indices in patients with various health conditions (1-4). Recent studies demonstrate that cyanidin 3-glucoside, a prominent dietary anthocyanin, ameliorates atherosclerosis in apoE-/- mice via microbiota-dependent conversion to protocatechuic acid (PCA) (5-7). Furthermore, in oxidized LDL-stimulated human vascular endothelial cells PCA and its conjugates (i.e., methyl, sulfate, glucuronide) lower expression of pro-inflammatory mediators, including Vascular Cell Adhesion Molecule 1 (VCAM-1) and Interleukin 6 (IL-6) (8). However, there is wide variation in the plasma levels of PCA and PCA-degradation products resulting from consumption of anthocyanins, even among healthy subjects (5, 9, 10), and anthocyanin absorption, metabolism, excretion and transit to the colonic microbiota evidently vary considerably (10). Doubtless this variation reflects numerous factors, often not well controlled, ranging from anthocyanin form (monomeric, polymeric), source complexity and formulation (single compound or mixture, liquid or dry), dosage type and kinetics (single bolus or sustained feeding), host condition (fed or fasting subjects) and metabolic capabilities (gut transit time, metabolite uptake and excretion), analytical extraction recoveries and measurements, as well as microbiota composition differences. We are using well-defined environmental conditions (i.e., gnotobiotic mice, defined diets and anthocyanin sources), sequencing and biochemical approaches to dissect the contributions of gut microbes on the beneficial effects associated with anthocyanin consumption.

These studies will enable the discovery of next-generation probiotics that could be given together with the flavonoids to maximize their effect. As we approach an era of personalized nutrition, our studies will help better inform how to make dietary recommendations for treatment/prevention of CVD that are matched with the metabolic potential of the patient's gut microbiome.

Key words: anthocyanins, microbiota, protocatechuic acid

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Andre Marette, Ph.D.

Laval University

Berries to fight the metabolic syndrome: what are the mechanisms?

Dr. André Marette graduated from Laval University in 1990 with a PhD in Physiology and Endocrinology. He his currently full professor in the department of Medicine and Scientific Director of the Institute of Nutrition and Functional Foods at Laval University, Québec, Canada. Dr. Marette is an international expert on the pathogenesis of inflammation, type 2 diabetes and cardiovascular diseases in obesity. His research in the areas of insulin action and insulin resistance, and the mechanisms of inflammation, has advanced the understanding of the cellular/molecular defects leading to diabetes and opened new possibilities for nutritional and pharmacological therapeutic interventions.

He has published over 170 papers and reviews in high-impact journals and he received several national and international research grants. Dr. Marette has received several awards including the Charles Best Lectureship Award of the University of Toronto, in recognition for his outstanding contribution to diabetes research. Dr Marette has organized a number of national and international meetings and symposia and has been invited to speak at more than a 150 national and international meetings.

Berries to fight the metabolic syndrome: what are the mechanisms?

The worldwide epidemic rise in type 2 diabetes (T2D) and cardiovascular disease (CVD) calls for novel treatments of these obesity-linked diseases. Visceral obesity is also associated with the development in nonalcohol fatty liver disease (NAFLD), which can lead to liver cirrhosis and cancer. Current pharmacological treatments for T2D and NAFLD have limited efficacy or unwanted side effects. I will present growing evidence that the gut microbiota is a key determinant of diet-induced obesity and T2D. I will show that new polyphenol-rich extracts from cranberry and other wild berries can protect against obesity-linked inflammation and alleviate T2D and NAFLD in high fat-fed mice. This is associated with a shift in the gut microbiota and in particular the increased abundance of the mucin-degrading bacterium *Akkermansia muciniphila*. Increase in the *Akkermansia* population may therefore contribute to the anti-inflammatory and beneficial effects of fruit polyphenols in obese mice. Finally I will present recent data showing that dietary supplementation with a polyphenol-rich supplement from strawberry and cranberry extracts improves insulin sensitivity in overweight and obese insulin-resistant men and women.

Simone Gugliemetti, Ph.D.

University of Milan

The role of microbes in the relationship between berries and human health

Guglielmetti graduated cum laude in Food Science and Technology at the University of Milan in 2001. In February 2005, he got the PhD degree in Food Biotechnology. In January 2005, he began working with a permanent position as researcher at the University of Milan. Since March 2015, he is Associated Professor at the Department of Food, Environmental, and Nutritional Sciences (University of Milan). In March 2015, he got a 5-years position as Adjunct Professor at the Tampere University of Technology (Finland).

Scientific activity

Guglielmetti is author of more than 50 publications international journals with impact factor, 5 international patents in the field of food science and microbiology, and 2 co-authorships in international scientific books. He is member of the group of coordination for the drafting of research priorities for the League of European Research Universities (LERU) within the Strategic Research Agenda Joint Programming Inittiative (JPI) – A healthy diet for a healthy life (2012, .PDF).

Research expertise

The primary research interest of prof. Guglielmetti deals with the study of food, agricultural, intestinal and probiotic bacteria. The main research topics of prof. Guglielmetti are:
development of new functional foods and microbial biotransformations for the food industry.
construction of molecular tools for the genetic modification of bacteria.
identification and characterization of the molecular determinants of adhesion and immunomodulation of probiotic and intestinal bacteria.
study of the impact of food on the microbial ecology of human intestinal tract and vaginal mucosa.

The role of microbes in the relationship between berries and human health

Berries are among the most commonly recognized health-promoting food due to their high content of polyphenols (especially anthocyanins), micronutrients, and fiber. The benefits of berries on human health span from reducing the incidence of cardiovascular disease to the prevention of obesity (Slavin and Lloyd, 2012). Such healthy activities of berries are associated to microorganisms at various levels. Specifically, microbes participate to the relationship between berry and human health principally at the following four levels:

1-Berries influence intestinal microbial ecology. Several studies demonstrated the ability of berries to change the relative abundance of specific bacterial taxa and the levels of short chain fatty acids in the gut; for instance, wild blueberries were shown to increase bifidobacteria in a human intervention study (Vendrame et al., 2011; Guglielmetti et al., 2013); in addition, blackcurrants were demonstrated to increase lactobacilli and decrease clostridia (Molan et al., 2010) determining higher intestinal concentrations of propionic and butyric acids in the rat intestine (Jakobsdottir et al., 2013); as a final example, ellagitannin-rich berries such as raspberries and cloudberries have been shown to increase the intestinal abundance of butyrate producing bacteria in human subjects with symptoms of metabolic syndrome (Puupponen-Pimiä et al., 2013). The ability of berries to modulate the intestinal microbiota seems principally associated to the high presence of fiber and polyphenols. In this context, anthocyanins, which are naturally present in berries as glycosides, are of particular interest. In fact, it has been hypothesized that anthocyanin glycosides may act as prebiotic molecules, i.e. nondigestible food ingredients that benefit the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Anthocyanin glycosides may transport the covalently bound sugar molecules into the colon where certain bacteria expressing specific glycosyl hydrolases, such as bifidobacteria and lactobacilli, may use the sugar moiety as carbon and energy source (Vendrame et al., 2011; Guglielmetti et al., 2013; Boto-Ordóñez et al., 2014; Faria et al., 2014).

2-Polyphenols in berries are metabolized by human microbiotas. The action of gut microorganisms on polyphenols leading to the production of metabolites with diverse physiological relevance has been also analyzed in the recent years (Etxeberria et al., 2013). Only a minor quantity of berry polyphenols (5–10%) is absorbed, and most part is extensively metabolized by oral and intestinal bacteria to low-molecular weight metabolites (e.g.: benzoic acids, phenylacetic acids, and phenylpropionic acids). Such microbial catabolites of polyphenols have been detected in many organs of the human body (including the brain) and have been proposed to contribute significantly to the biological activities ascribed to berry polyphenols (Williamson and Clifford, 2010).

3-Berries may inhibit specific microbial pathogens. Numerous studies demonstrate the antimicrobial properties of berries, which are principally associated to phenolic compounds. Particularly, berry extracts may inhibit intestinal pathogens such as Staphylococcus aureus, Salmonella enterica and Escherichia coli (Puupponen-Pimia et al., 2001; 2005).

4-Berries cooperate with microbes in the modulation of the immune system. Berries display immunomodulatory activities principally due to the direct activity of anthocyanins (Taverniti et al., 2014). However, recent studies demonstrate that berry anthocyanins may also efficiently influence host immune system by enhancing immune cell responses to bacteria, and particularly probiotics (Frøkiær et al., 2012). These data suggest that the combined use of probiotics and berries may be a promising approach for the development of novel improved immunomodulatory strategies.

In conclusion, microbiology is emerging as a discipline that can profoundly contribute to the understanding of the health promoting properties of berries. Nonetheless, for certain experimental approaches research is still at a preliminary phase; particularly, human interventions studies with a proper trial design (e.g. cross-over) coupled with modern microbiomic techniques are needed in order to appropriately elucidate the actual impact that different berries may have on human intestinal microbial ecology.

Key words
Intestinal microbial ecology, prebiotics, probiotics, anthocyanins, bifidobacteria

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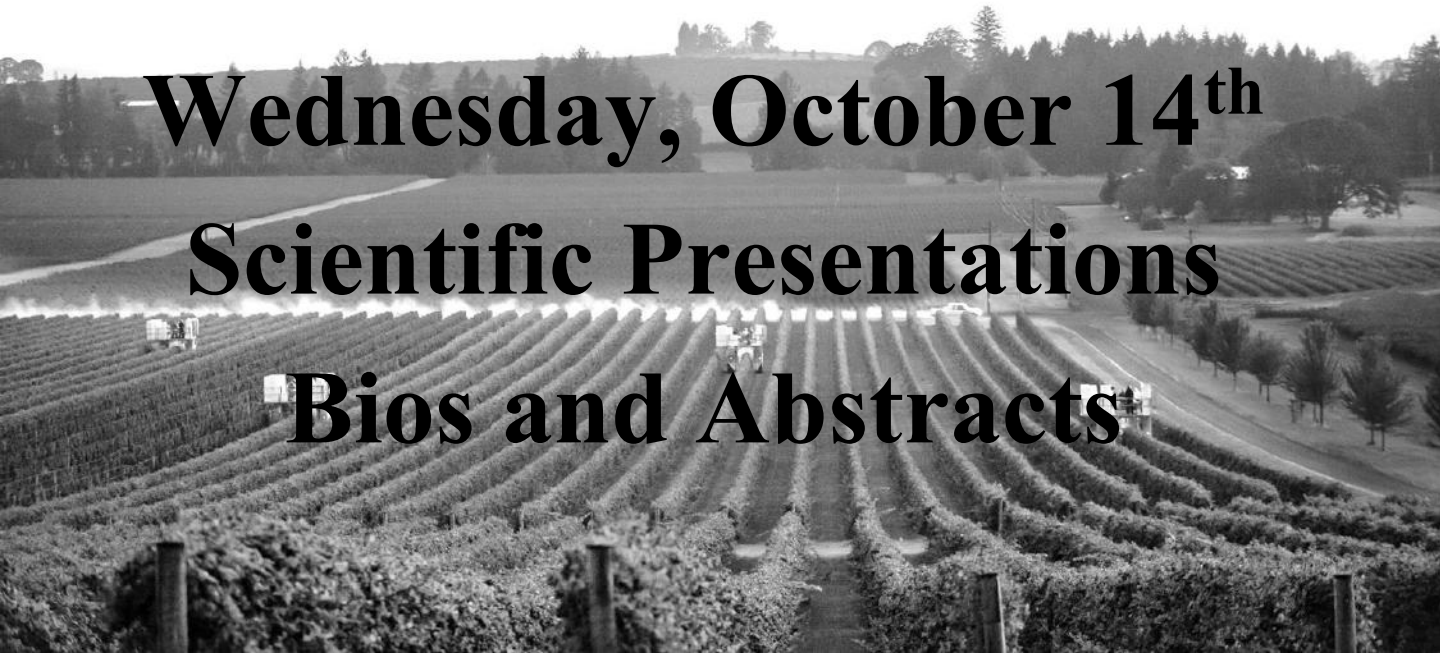


Wednesday, October 14th
Scientific Presentations
Bios and Abstracts

Berries and Brain Aging

Barbara Shukitt-Hale, Ph. D.

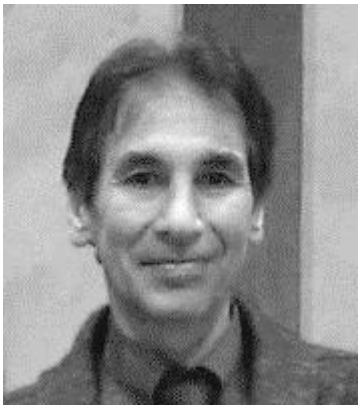
Session Chair
USDA/Tufts University

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.



Robert Krikorian, Ph.D.

University of Cincinnati

Berry supplementation to mitigate neurocognitive decline

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 supplementation to mitigate neurocognitive decline

Neurocognitive decline with aging in conditions such as Alzheimer's disease represents a substantial public health concern that produces suffering in patients and caregivers and enormous expenditure of health care resources [1]. Effective medical treatment for dementia is not yet available [2]. However, attention has begun to shift to preventive measures, and nutritional intervention represents a potentially potent approach to mitigate risk for late life dementia.

A large body of preclinical research has indicated that berry fruit supplementation can improve cognitive performance and several aspects of neural function in aged animals [3,4]. In addition, relatively few human studies in older adults have provided preliminary evidence of enhancement of memory and brain function [5,6]. However, there are methodological concerns and questions regarding the developmental stage at which preventive interventions should be introduced for optimal benefit. We will review findings of our most recent human berry trials in older adult samples with varying risk for AD, examining the effects on cognitive performance and brain function. While such moderate-term intervention trials have limitations, they suggest the possibility that nutritional approaches, when introduced in advance of dementia, may contribute to risk reduction.

Key words: Aging, dementia, berry supplementation

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Carol Cheatham, Ph.D.

University of North Carolina

The effect of blueberry consumption on cognitive abilities in 65- to 79-year-olds: a 6-month randomized controlled trial

Dr. Cheatham is a developmental cognitive neuroscientist and a member of the Nutrition & Brain Development Team at the University of North Carolina – Chapel Hill Nutrition Research Institute (NRI) on the North Carolina Research Campus. At the NRI, Dr. Cheatham is studying the effects of nutrients (e.g., fatty acids, choline, iron, zinc, antioxidants) on the development and functioning of the hippocampus and frontal lobes, brain structures that are integral to the formation and retrieval of memories and to higher-order cognition. She uses both cognitive testing and an electrophysiological technique known as event-related potentials in her work with adults, as well as behavioral assessments in her studies with children.

Dr. Cheatham earned her Ph.D. in Child Psychology with an emphasis in Neuroscience in September 2004 at the Institute of Child Development, University of Minnesota – Twin Cities (rated number one in the nation by US News & World Report) with Patricia Bauer and Megan Gunnar, both internationally renowned in their field. During her tenure at the Institute, she studied the development of memory and attention with Dr. Bauer, while simultaneously studying the effects of stress and social support on memory development with Dr. Gunnar.

She first became interested in the interplay between nutrition and brain development during her work with the chair of her dissertation committee, Dr. Michael Georgieff, a leading neonatologist who studies the effects of iron intake on brain development. Even though her dissertation did not have a nutrition component, the mentoring she received from Dr. Georgieff was invaluable for the understanding of the effects of nutrition on the brain. In addition, she began to appreciate the value of interdisciplinary ventures and came away with a desire to seek collaborative opportunities that cross traditional lines. She subsequently accepted a position on an interdisciplinary project at the University of Kansas Medical Center (KUMC) exploring the effects of docosahexaenoic acid (DHA), a fatty acid, on cognitive development. Dr. Cheatham views interdisciplinary work as a pathway to a cohesive picture of brain development and functioning.

In her recent research, Dr. Cheatham hypothesizes that DHA's effects on the ability of the brain to process information or even the ability of the brain to utilize DHA when it is present may be differentially affected by background diet (e.g., the total fat composition of the diet) and the organism's history (e.g., expression of genes). She is assessing the effects of omega-3 fatty acids on declarative memory using behavioral and electrophysiological (event-related potential) paradigms. Important to her work at the Nutrition Research Institute is the ability to classify participants by single nucleotide polymorphisms (snp) in genes that are involved in the fatty acid metabolism process and are possibly introducing a confound into the data as people of a certain genotype metabolize fatty acids more readily than others.

Dr. Cheatham's goal is to elucidate factors surrounding hippocampal development in children and senescence in adults. She believes that a focus on the nutritional aspect of brain research is key to discovering possible interventions that would help improve brain development and slow cognitive decline, giving every child and adult a chance to maximize their own cognitive abilities.

The effect of blueberry consumption on cognitive abilities in 65- to 79-year-olds: a 6-month randomized controlled trial

As humans age, they experience gradual cognitive decline characterized by a general slowing of processing and a decrease in memory abilities. At least in part, this dysfunction is related to a decrease in neurogenesis in the hippocampus – plasticity that is integral to memory formation. Blueberries contain anthocyanins. Previous research has shown that plasticity is increased in blueberry-supplemented aged rats (Casadesus et al., 2004). The anthocyanins are increased in the brain of blueberry-fed aged animals, and importantly, the increase is found in the neural areas related to memory and processing (Andres-Lacueva et al., 2005). It is still unclear what effect blueberry consumption will have on the brain function in a human sample. Thus, this clinical trial was designed to determine the effect of dietary intake of blueberries on cognitive senescence in humans. We tested whether adults with mild cognitive decline, who consume blueberries, would experience a slowing of cognitive decline in processing speed and memory abilities, as measured by a battery of standardized tests and in an electrophysiology paradigm, and as compared to those who consume a placebo.

The double-blind randomized controlled trial was conducted with adults aged 65-79 years (n=133) who were screened for general health, dietary habits, and cognitive abilities. Mild cognitive decline was defined as 1.5 standard deviations below the aged norm on the Montreal Cognitive Assessment (MoCA); those who scored within typical ranges were enrolled in a reference control group (n=88 and 45, respectively). The mild cognitive decline group was randomized into two arms: blueberry and placebo. Participants were asked to consume the equivalent of two cups of freeze-dried and pulverized (powdered) blueberries daily for 6 months and to maintain a consistent diet and exercise routine. Compliance was assessed by a count of unused, returned powder packets.

Cognitive abilities were tested using the Cambridge Neuropsychological Test Automated Battery (CANTAB), the MoCA, the Wechsler Adult Intelligence Scale (WAIS), and an electrophysiology paradigm known as event-related potentials. Tests were administered at baseline, 90 days, and 180 days.

To determine whether diets supplemented with blueberries slow cognitive decline, performance in the supplemented groups will be compared to performance of the placebo group. Cognitive scores will be entered into a multivariate analysis of covariance (MANCOVA) with group (active, placebo) as the between-subjects variable and covariates as needed. We expect that at the 6 months endpoint, the supplemented group will perform better on the cognitive tasks than will the placebo group. Preliminary results will be presented.

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Marshall Miller, Ph.D.

Tufts University

Effects of berry supplementation on mobility and cognition among older adults

Dr. Miller is a research psychologist in the Neuroscience and Aging Laboratory at the USDA-ARS Human Nutrition Research Center on Aging (HNRCA) at Tufts University. He received his Ph.D. in Psychology from Tufts University in 2014. He is a recipient of the Emerging Leaders in Nutrition Science award (American Society for Nutrition, 2015), Second Place Paul F. Glenn award (American Aging Association, 2014), and Distinguished Graduate Student Award (Psi Chi, 2008).

Before coming to the HNRCA, he was a post-baccalaureate fellow in the Laboratory of Experimental Gerontology at the NIA Gerontology Research Center. Dr. Miller's current research focuses on aging and its effects on the brain and behavior. His recent studies have used pre-clinical and clinical approaches to investigate dietary interventions for age-related cognitive decline.

Effects of berry supplementation on mobility and cognition among older adults

Americans are now living longer than ever before; as a result, the number of people over the age of 65 is expected to double by 2050. During this same period, the number of people living with dementia is expected to almost triple. Even in the absence of specific neuropathology such as Alzheimer's or Parkinson's disease, age-related neurodegeneration leads to measurable declines in cognitive and motor function. Despite more likely causes of death, dementia is a chief health concern among older adults due to the loss of dignity, identity, and independence. While its causes are not yet entirely understood, neuroinflammation and oxidative stress are thought to underlie many of the processes and pathologies that lead to dementia.

Epidemiological studies have found that diets rich in berry fruit, such as blueberries and strawberries, are associated with lower rates of age-related cognitive decline. Berry fruit contain a variety of neuroavailable phytochemicals, which can alter neuronal morphology and signaling as well as reduce neuroinflammation and oxidative stress. When aged rats consumed diets containing 2% blueberry or strawberry for 2 months, improvements in spatial working memory, balance, and coordination were observed relative to controls. Therefore, dietary interventions with berry fruit may represent a promising approach to slowing or preventing age-related neurodegeneration.

Recently our lab has sought to translate the beneficial effects of berry fruit interventions from rodent models to older human populations. An initial study established methods for assessing age-related functional decline among older adults using tests that parallel those used in rodent models of aging. Seventy six healthy adults, between the ages of 21 and 75, completed a 1 hour mobility and cognition assessment. Increases in postural sway during quiet standing, decreases in gait speed, and impairments in spatial learning and memory were observed with age.

To determine whether dietary intervention with blueberry could reverse age-related motor and cognitive decline among older adults, 38 healthy men and women between the ages of 60 and 75 years were recruited into a randomized, double-blind, placebo-controlled trial where they consumed 24g/d of freeze-dried blueberry powder (~1 cup/d whole blueberry) or a blueberry placebo powder for 90 days. Participants were tested at baseline and again at 45 and 90 days. Although no improvements in mobility were observed, participants that consumed blueberry made fewer repetition errors in a verbal learning task and fewer errors in a test of mental flexibility over the course of the study, relative to those in the placebo group. We are currently conducting a similar study involving 39 older men and women to determine whether dietary intervention with 24g/d strawberry (~2 cups/d whole strawberry) for 90 days can improve age-related changes in motor function and cognition. These findings provide evidence that supplementing older adults' diets with berry fruits can improve cognition even during otherwise healthy aging.

Key Words: Aging, cognition, mobility, inflammation, oxidative stress, berry fruit

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Claire Williams Ph.D.

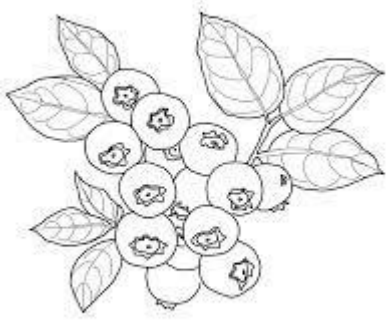
University of Reading

Effects Of Flavonoid-Rich Blueberry Interventions On Cognitive Behaviour In 7-10 Year Old Children

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 BLUEBERRY INTERVENTIONS ON COGNITIVE BEHAVIOUR IN 7-10 YEAR OLD CHILDREN

In recent years the desire to understand the relationship between nutrition and behaviour has grown substantially. In particular, the impact of nutrients and other food substances on brain function, cognition and mental performance has received rigorous scientific research attention as well as media interest. As a result there is a substantial body of evidence from human intervention studies demonstrating that the consumption of flavonoids is associated with benefits in cognitive function (for a review see Lamport et al. 2012). Specifically, our laboratory has shown that both flavanol-rich foods (such as cocoa) and anthocyanin-rich foods (such as blueberry) are capable of promoting cognitive improvements in both animal models and adult human studies (for a review see Rendeiro et al. 2012). These effects on cognition, if translated to children and adolescents, would be of clear practical and theoretical importance, particularly in an academic context.

Recent work from our laboratory has recently started to address this question. Using a cross-over design fourteen 8-10 year old children consumed either a flavonoid-rich blueberry drink or matched vehicle/placebo before completing a battery of cognitive tests. This age group were chosen as children at this age are in a stage of development where there is a spurt of growth in the frontal lobes of the brain, which coincides with development in executive function (such as cognitive flexibility, goal setting and information processing etc). Children supplemented with the blueberry drink demonstrated significant improvements in the delayed recall of a previously learned list of words (Whyte & Williams, 2014).

In a follow-up study, we have described a double-blind cross-over dose and time course study examining the cognitive effects of blueberry supplementation in the same age group. Here, on three occasions children consumed either a placebo (vehicle), low- or high-dose blueberry drink and performed a battery of cognitive tests (targeting executive function and memory processes) at baseline, and 1.15, 3 and 6 hours post-dosing. Importantly, we replicated the cognitive benefits of flavonoid treatment seen in our first study, with significant blueberry-related improvements in immediate word recall and better delayed word recognition in a verbal learning and memory task, as well as improved accuracy on cognitively demanding incongruent trials in an executive function task. Importantly, across all measures, cognitive performance improved, consistent with a dose-response model, with best performance seen following high-dose blueberry drink and worst following the vehicle drink. This is the first multi-dose double-blind study to show positive cognitive effects following flavonoid intervention with school-age children and the benefits to attention and memory we report may prove particularly beneficial in an educational setting.

In addition to our initial trials, we will discuss the findings of recent clinical trials from our laboratory investigating the effects of flavonoid supplementation on the development of language and literacy skills in typically developing children and children with language and literacy difficulties.

Keywords

Flavonoid, Anthocyanins, Blueberry, Children, Cognitive, Memory, Attention

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Berries and Cancer

Ramesh Gupta, Ph. D.

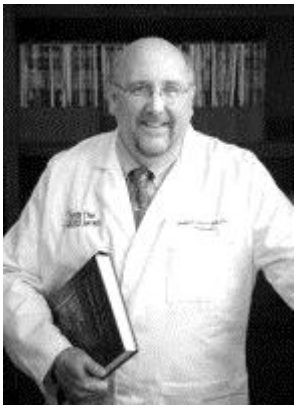
Session Chair
University of Louisville

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 32P-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.



Steve Clinton, Ph.D.

Ohio State University

Black Raspberries and Cancer Prevention: A "Crops to the Clinic" Transdisciplinary Strategy at The Ohio State University

Steven K. Clinton, MD, PhD is the John B. and Jane T. McCoy Chair in Cancer Research at the OSU Comprehensive Cancer Center and a Professor of Internal Medicine in the Division of Medical Oncology at The Ohio State University. He trained for his M.D. and Ph.D. at the University of Illinois in Urban Champaign followed by Internal Medicine internship and residency at the University of Chicago. He proceeded with Medical Oncology training at the Dana-Farber Cancer Institute and Harvard Medical School where he remained on faculty for nearly a decade.

Dr. Clinton joined The Ohio State University in 1998. He is Director of the Prostate and Genitourinary Oncology Program at The James Cancer Hospital and Solove Research Institute. Under his leadership, the clinical program is a national leader in accrual to therapeutic and prevention clinical trials. The program provides integrated multidisciplinary oncology care to patients throughout central Ohio and for national and global referrals.

Dr. Clinton also serves as the Program Leader for Molecular Carcinogenesis and Chemoprevention at The Ohio State University Comprehensive Cancer Center. The focus of this program, involving over 40 faculty, is to elucidate the fundamental mechanisms underlying the development of cancer and to define prevention strategies. A major strength of the program is the "crops to the clinic" research agenda that integrates the efforts of scientists in agriculture, food science, and nutrition with clinical investigators to conduct novel human clinical trials at The James.

Dr. Clinton is very active in bionutrition research at OSU. He serves as Associate Director for the campus wide Center for Advanced Functional Foods Research (CAFFRE) and the OSU Food Innovation Center (FIC), programs dedicated to the development of novel food products and their evaluation for promoting health outcomes and improving global nutrition. He provides service to many national organizations including the American Association for Cancer Research, the American Society of Nutrition, and the American Society for Clinical Oncology.

He recently served the National Academy of Sciences and Institute of Medicine as a member of the Dietary Reference Intake Committee for Vitamin D and Calcium. He is currently serving on the World Cancer Research Foundation / American Institute for Cancer Research (WCRF/AICR) Continuous Update Project committee. He is a member of the Advisory Committee for the United States Department of Health and Human Services and United States Department of Agriculture Dietary Guidelines for America 2015 Report. Dr. Clinton's research activities focus on many aspects of diet, nutrition, and cancer; primarily focusing upon prostate and other genitourinary cancers. The research efforts include metabolic epidemiology, clinical intervention trials, as well as basic laboratory studies of cellular and molecular biology resulting in over 200 scientific publications, reviews, and book chapters.

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Black Raspberries and Cancer Prevention: A "Crops to the Clinic" Transdisciplinary Strategy at The Ohio State University

Overview: A plant-based dietary pattern rich in fruits, vegetables, and whole grains is the foundation for evidence-based dietary guidelines for cancer prevention. The contribution of specific phytochemical-rich plant food items is less certain, and one interdisciplinary effort at The Ohio State University is focused upon the hypothesized anti-cancer properties of black raspberries (BRB) (Rubus Occidentialis). Our investigations emphasize a transdisciplinary "crops to the clinic" strategy that links agricultural and food scientists with basic and translational cancer researchers to develop and test novel BRB food products for cancer prevention while examining supportive mechanisms of action in multiple model systems.

Black Raspberry Food Products: BRB are a source of many bioactive polyphenols. The two most predominant classes of polyphenols in BRR are anthocyanins and ellagitannins. We have found that the chemical pattern of BRB components is modulated by genetics (cultivar), growing conditions, and ripening, which can impact resultant bioactivity (Johnson et al, 2011; Paudel et al, 2014). Thus, careful characterization of a food product for clinical trials by HPLC-MS or other means is essential to precisely define exposure. Our concept is that unique food products can be strategically designed to optimally target cancer risk in specific organs. For example, BRB confection and nectar products have been designed to enhance direct exposure of the oral mucosa and esophagus to bioactives (Gu et al, 2014; Gu et al, 2015). Phytochemical retention during processing has also been quantitated. Furthermore, novel products can be developed that enhance systemic absorption or metabolism by the colonic microbiota.

Human Polyphenol Exposure: In order to proceed with informative clinical trials, we have conducted a study to define polyphenol intake in Americans consuming a typical diet. We collected food consumption data and employed the Polyphenol Explorer® database (Neveu et al, 2010) to assess exposures to total
and specific types of polyphenols. Estimated total polyphenol intake was 1571± 947 mg/day, with coffee/tea beverages (1069 ± 966 mg/day) as the largest contributors. These estimates are consistent with
those observed in the European population (Zamora-Ros et al, 2015). Intake of one specific class of polyphenols, ellagitannins, was 12 ± 13 mg/day (range: 0-35mg/day). We also have developed low-
polyphenol and low-ellagitannin dietary plans and demonstrated that humans can easily adhere to these for 4 wks. Adherence to a low polyphenol diet was 95%, resulting in an 85% drop in total estimated dietary polyphenol consumption compared to the regular intake group. Adherence to a low ellagitannin
diet was 97%, resulting in a 99% drop in ellagitannin consumption (50% less polyphenols). We have demonstrated the utility of these diets to serve as a control, to reduce background polyphenol exposure
and blood or urinary metabolites, and to improve the ability to study polyphenol metabolism after interventions with specific polyphenol-rich foods.

Human Studies of Novel BRB Food Products: We recently completed a 4 wk study in men assigned to consume BRB-nectar or BRB-confections, each at two daily doses corresponding to10 or 20g of freeze- dried BRB powder. These men experienced no adverse events, and their adherence to the study products was excellent (>99%). Elligitannin metabolites (urolithin A, urolithin B, urolithin C, urolithin D, and di- methyl ellagic acid) concentrations in the urine greatly increased and were strongly reflective of the BRB dose provided. These fully characterized food products are ideally suited for human studies of bioactivity and/or metabolism.

Our preclinical studies support the anticancer activity of BRB in oral carcinogenesis (Warner et al, 2014), perhaps via impacting inflammatory and immune pathways (Mace et al, 2014). In a study of patients with oral cancer, we examined confection-type products for their ability to impact biomarkers in oral cancers
and the oral mucosa (exposure 13.9 ± 1.3 days). For example, following BRB administration, the expression of pro-survival genes (AURKA, BIRC5, EGFR) and pro-inflammatory genes (NFKB1, PTGS2) were significantly reduced. BRB phytochemicals cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside were detected in oral tissues and oral cancers, demonstrating that bioactive components were successfully reaching targeted oral tissues. Ongoing human studies address the complex interactions between BRB phytochemicals, the oral microbiome, and tobacco exposure during oral carcinogenesis.

Experimental Esophageal Carcinogenesis: Feeding of BRB inhibits experimental esophageal squamous cell carcinogenesis (Chen et al, 2006; Stoner et al, 2007) in association with inhibition of proliferative signaling and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), among others. Our current studies suggest that BRB feeding is more potent in prevention of experimental esophageal carcinogenesis than chemopreventive agents that inhibit iNOS or COX-2. Our findings support the continued evaluation in human studies targeting squamous esophageal carcinogenesis.

Experimental Prostate Carcinogenesis: Control and TRAMP mice were fed diets with 10% BRB
powder for 6 wks after weaning to examine the impact on gene expression patterns in early
carcinogenesis. RNA-seq studies show that BRB feeding impacted expression of genes involved in cancer related pathways in both normal and TRAMP mice. HPLC-MS studies show that urinary BRB
ellagitannin metabolites, urolithins, were absorbed and detected in plasma, liver, and the prostate. These
findings support the hypothesis that BRB metabolites have direct and/or indirect impact on the prostate.

Experimental Colon Carcinogenesis: Substantial progress has been made examining the potential role of BRB phytochemicals for the inhibition of experimental colon cancer both in vitro (Paudel et al, 2014) and in vivo (Bi et al, 2010; Harris et al, 2001), with additional supportive data from small human trials (Wang et al, 2014). We are currently evaluating the colon microbiome as one potential component of the process whereby the BRB food products may impact colon carcinogenesis. We have observed that BRB feeding at 10% of the diet alters the composition of the microbial flora in the colon mucosa and in the colon contents in unique patterns.

Summary: BRB contain a rich profile of phytochemicals demonstrating bioactivity in a variety of in vitro, rodent, and human studies. Specific chemical constituents and relevant metabolites are being actively investigated for mechanisms of action. Horticulture and food science contributes significantly to developing consistent food products for human studies that are safe and provide excellent compliance. Ongoing studies are examining the absorption, metabolism, and bioactivity in order to guide definitive human studies with clinically relevant endpoints. This "crops to the clinic" approach, integrating investigators across disciplines, is a model for impactful translational research that reduces cancer burden.

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Farrukh Aquil, Ph.D.

University of Louisville

Cancer prevention and therapeutic efficacy of berry bioactives

Farrukh Aqil, Ph.D., is an Assistant Professor of Medicine. He received his doctorate in Microbiology from and has extensive experience of over 12 years in phytochemistry, microbiology, cancer biology and cancer prevention. Prior to moving to the as a Postdoctoral Associate in the Cancer Chemoprevention Group, he has a faculty in the Department of Biotechnology.

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 has participated in many conferences and presented his work in the form of 60 abstracts/oral presentations. Dr. Aqil has authored or co-authored over 45 articles in peer reviewed journals, has 12 book chapters and has edited 4 books. He is an as associate editor in International Research Journal of Microbiology and serves as peer reviewer for more than 30 journals.

Cancer Prevention and Therapeutic Efficacy of Berry Bioactives in Pre-clinical Studies

Farrukh Aqil[1, 2], Jeyaprakash Jeyabalan[1], Ashish K. Agrawal[1], Radha Munagala[1, 2] and Ramesh Gupta[1, 3]

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Berries have been utilized effectively for their chemopreventive and therapeutic potential against several cancers. Our own studies have shown significant inhibition of breast cancer in the rat model by both Berkley blueberry and black raspberry, indicating that berries demonstrate chemopreventive efficacy beyond the GI tract. Recently we demonstrated both chemopreventive and therapeutic efficacy of high anthocyanin-containing highbush blueberry powder against estrogen-mediated breast cancer.

In order to demonstrate the effectiveness of blueberry against lung cancer, we tested BB diet in *in vivo* tumor xenograft model. Diets supplemented with 2.5-7.5% were investigated against the growth of the A549 cancer cell xenografts. At the end of 6 weeks, animals provided diet supplemented with different doses of highbush berry showed significant inhibition of the tumor growth compared to control diet.

The anticancer activities in berries are attributed to polyphenolics including anthocyanins. Our initial studies indicated significantly higher antiproliferative activity of anthocyanins-enriched berry extract compared with extract prepared at anthocyanidin level against human H1299 lung cancer cells. We then developed methods to isolate pure anthocyanidins in large quantities and showed significant therapeutic activity against lung cancer in both cell culture and animal studies. The combination of suboptimal equimolar concentrations of anthocyanidins synergistically inhibited growth of two aggressive non-small-cell lung cancer cells *in vitro* and *in vivo*, with minimal effects on non-tumorigenic cells.

The native mixture of bilberry-derived anthocyanidins also showed high antiproliferative activity against both drug-sensitive (A2780) and drug-resistant (A2780/CP70 and OVCA432) ovarian cancer cells *in vitro*. The anthocyanidin mixture also showed dose-dependent inhibition of ovarian cancer (A2780) cell tumor xenograft in nude mice. New strategies have been developed to enhance efficacy of bilberry anthocyanindins, and our preliminary data indicate that the anthocyanidins when encapsulated in biological nanoparticles (milk-derived exosomes) can significantly enhance therapeutic efficacy of the anthocyanidins and/or reduce dose against the growth of lung and ovarian cancer.

Together, our data demonstrate that blueberry/bilberry and their bioactives particularly anthocyanidins have high therapeutic potential against various cancers and advance our understanding in search of plant therapeutics.

Key words: Blueberry, Anthocyanidins, Breast cancer, Lung cancer, Ovarian cancer, Exosome

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Laura Kresty, Ph.D.

Medical College of Wisconsin

Summary of Clinical Trials Utilizing Black Raspberries to Target Premalignancy or Cancer

Laura Kresty, Ph.D., M.S., is an Associate Professor of Medicine at the Medical College (MCW) of Wisconsin Division of Hematology and Oncology, specializing in Cancer Prevention. Her laboratory is focused on evaluating preventive agents and novel strategies for targeting cancers of the esophagus and head and neck. Dr. Kresty's ongoing research includes evaluating the cancer inhibitory potential of cranberry constituents, vitamin D and investigating energy excess as it relates to esophageal adenocarcinoma risk. Her laboratory is also collaborating on investigations focused on novel imaging technologies to detect early epithelial and sub-epithelial esophageal changes for more rapid evaluation of chemopreventive agents.

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. She 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 program of research and served as Director for the Doctorate in Epidemiology Program. Dr. Kresty joined the MCW in January 2013.

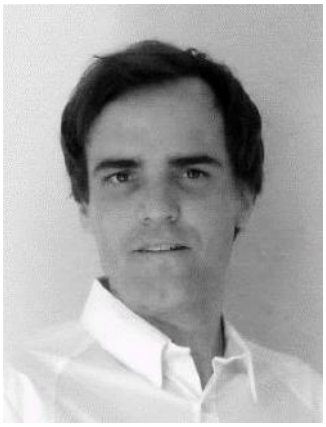
Dr. Kresty serves as a peer reviewer for multiple journals in her field, has over 40 peer-reviewed research articles and book chapters, and has delivered more than 35 invited talks throughout the world. She is NCI funded.

A Summary of Clinical Trials Utilizing Black Raspberries to Target Premalignancy or Cancer

Black raspberries (BRB) have been reported to inhibit a broad range of cancers in preclinical models, including in vivo models of oral, esophageal, colon, breast and skin cancer. These promising preclinical results have led to clinical evaluations in cancer patients or patients at increased risk for cancer development, including those with Barrett's esophagus and FAP and ongoing studies targeting ulcerative colitis, smokers and prostate cancer patients.

To date completed published studies evaluating BRB in the clinical setting report positive effects on preneoplastic lesions or cancers of the head and neck, esophagus and colon. The positive effects of black raspberries in clinical evaluations include: anti-proliferative effects; activation of pro-cell death pathways; histologic regression of oral intraepithelial neoplasia associated with improved histologic grade and significantly reduced LOH at tumor suppressor gene associated loci, reduced suppression of genes linked to RNA processing, growth factor recycling, reduced COX-2 levels; in the colon, inhibition of FAP-associated polyp progression, demethylation of known tumor suppressor genes (Wnt pathway antagonists and p16) and improved plasma cytokine profiles (GM-CSF, IL-8); in Barrett's patients, increased tissue levels of GST-π, decreased lipid peroxidation/oxidative stress (urinary 8-isoprostane levels) and reduced cholesterol. In addition, other studies of obese patients have reported improved serum cytokine levels and lipid profiles (IL-6, TNF-α, cholesterol).

Results have not all been uniform across all studies or even within, most studies report a percentage of responders and non-responders within the cohort evaluated, likely reflecting the heterogeneity between subjects and the complexity of each cancer or precursor lesion. In addition, the mode, concentration, frequency and duration of black raspberry delivery has varied across studies also potentially contributing to divergent outcomes. Black raspberries have been administered as a freeze-dried powder resuspended in water prior to consumption (37 to 60 g/day), as a rectal suppository alone (1.4 g/day) or in combination with oral delivery (60 g/day) and as a topical agent (10% w/w) delivered in a bioadhesive gel in the oral cavity. Studies conducted in patients with FAP or oral premalignancy permitted relative direct delivery of BRB bioactive constituents to the target area. This has the advantage of requiring less product, increasing direct contact time and takes advantage of local metabolism compared with delivery of the resuspended freeze-dried powder. Still, common themes across studies emerged including that BRB have anti-inflammatory effects, reduce oxidative stress and restore tumor suppressive activity in a target specific manner resulting in regression of oral cavity and colon lesions. The precise dose and duration of BRB required for optimum cancer inhibitory effects remains to be elucidated, but results to date suggest lower concentrations may favorably impact lipid profiles, but potentially higher concentrations are required for tissue specific effects. In addition, studies lengths have ranged from 1 week to 9 months with data supporting that longer duration studies have greater impact. This presentation will summarize details of the current research, discuss advantages as well as challenges to the clinical application of utilizing BRB and suggest future approaches for targeting specific cancers or precursor lesions with BRB.



Luc Biedermann, Ph.D.

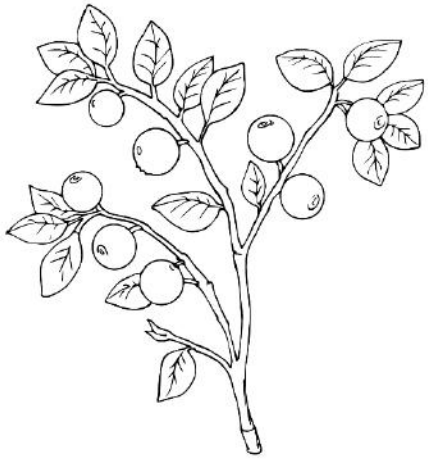
University Hospital of Zurich

Bilberry in IBD – just getting blue or getting better?

Dr. Luc Biedermann attended medical school in Basel, Switzerland. After board certification in internal medicine at the University Hospital Zurich, he specialized in gastroenterology (2nd board certification). He currently works as a senior physician at the division of Gastroenterology & Hepatology of the University Hospital Zurich (USZ) chaired by Prof. Michael Fried.

Among his main clinical and research interests are inflammatory bowel disease (IBD) and also celiac disease as well recurrent clostridium difficile colitis. Under the lead of Prof. Gerhard Rogler he works in his main clinical focus in the inflammatory bowel disease clinic of the University Hospital Zurich, a tertiary referral center for ambulatory and in-house care for IBD patients in Switzerland.

Dr. Biedermann is especially interested in aspects of intestinal microbiota, epidemiology and alternative treatment options in IBD. The possibility of providing patients new treatment options within clinical trials is an important component in the care of IBD patients in Zurich. The USZ IBD-clinic participated in a multitude of phase II and III studies, including smaller investigator initiated studies, either multi centre within Switzerland or single center. The topic of his talk will be centered around the results of a small pilot study on the effect of an anthocyanin-rich bilberry preparation in patients with ulcerative colitis.



Bilberry in IBD – just getting blue or getting better?

Keywords:
Ulcerative Colitis, Bilberries, Anthocyanins, Clinical Trial, Antioxidants, Nutrition, Complementary and Alternative Medicine

Anthocyanins (ACs) are a subdivision of flavonoids, and are responsible for the vivid blue, purple and red colours of plants such as in fruits, flowers and leaves, occurring in especially high abundance in red, blue and black berries including bilberries (*Vaccinium myrtillus*), but also in red wine, cereals and certain vegetables[1,2]. Due to their phenolic structure ACs show antioxidative capacity *in vitro* and *in vivo* as they are able to scavenge reactive oxygen species (ROS) [3]. Given their wide distribution, ACs have been frequently consumed as part of the daily human diet for hundreds of years. Over the past decade, interest in these phenolic compounds has dramatically increased due to accumulating evidence for beneficial effects in human health[1,4]. For instance, ACs are considered to reduce the risk of coronary heart disease by inhibition of the oxidation of low-density lipoprotein (LDL) [5]. Numerous studies have demonstrated their potential anticarcinogenic, anti-inflammatory, antimicrobial and antioxidant bioactivity. Furthermore, recent animal models and human pilot trials have suggested their role in protecting the gastrointestinal tract against diseases such as ulcerative colitis and certain cancers rendering them possible therapeutic agents either by itself or in conjunction with medical treatment regimens.

Inflammatory bowel disease (IBD) refers to a group of chronic inflammatory disorders of the gastrointestinal tract, with Crohn's disease (CD) and ulcerative colitis (UC) as their main representatives. UC is a chronic disease characterized by inflammation of the large intestine in various extensions, in contrast to CD rather involving the terminal segments of the small bowel. Diarrhea, often bloody, as well as abdominal cramps are among the most common symptoms of UC. Although the clinical course is highly variable, most patients suffer from recurrent flares and also the unpredictable course of their disease. An important hallmark of both disease states is chronicity (persistent, frequent, repeated or waxing and waning symptoms reflective of inflammatory activity) as well as a lack of causal medical treatment options to cure these diseases. About two thirds of all UC patients with mild to moderate disease activity can be successfully treated with mesalamine, which is an anti-inflammatory drug either used systemically (by mouth) and/or topically (by rectum) with only minimal side effects [6]. However, the group of patients that do not achieve a sufficient clinical response, represent a clinical challenge. Although there are a growing number of potential effective drugs to be used in these patients, the overall profile of these agents is far from being ideal. These drugs harbor a considerable risk of short- and long-term toxicity and side effects. Moreover, the costs of newer treatment options are very high, what especially represents a problem in less prosperous countries in the world. Most importantly, even the newest (and to some extent not yet approved) treatment options, do not work in all patients. Considering these points, it becomes obvious, that the development of supplemental medical treatment options, with favorable cost-benefit ratio and salutary side-effect profile represents an unmet clinical need in UC.

A high fraction of patients with IBD wish to apply or as a matter of fact use complementary and alternative medicines (CAM) [7–10]. A lack of sufficient long-term efficacy of established treatment options [9] as well as side effects [10] attributed to the use of these established therapeutic strategies are important reasons underlying the motivation of using CAM in patients with IBD. Despite the abundant use, of CAM there is a fundamental lack of high-quality studies to legitimate their place in the treatment algorithms for IBD.

Various previous investigations indicated a therapeutic potential of ACs in IBD, such as interruption of CD40-mediated pro-inflammatory signaling or inhibition of 5-lipoxygenase, a key enzyme for the biosynthesis of active leukotrienes [11]. In the presence of flavonoids monocytes release less TNF and IL-8 [12]. ACs also inhibit production of cyclooxygenase 2 [13] and activation of NF-kB [14]. NF-kB as well as TNF and IL-8 are key molecules mediating inflammation in IBD. This may be mediated by inhibition of proteasomal function [15].

In an in vivo model of colitis we induced acute and chronic dextrane sodium sulphate (DSS) colitis in Balb/c mice by 2.5% DSS in the drinking water. Mice fed with dried bilberries as AC source revealed a lower disease severity and reduced secretion of IFN-γ and TNF from mesenteric lymph nodes [16]. Dried bilberries also improved chronic DSS colitis. These results were in concordance with other investigations in animal models indicating a beneficial effect [17–19].We recently reported on the results of a subsequent uncontrolled small pilot study in humans investigating the therapeutic potential of an AC-rich bilberry preparation in patients with UC [20]. At the end of the 6 week treatment interval 63.4% of patients achieved remission, the primary endpoint, while 90.9% of patients showed a response. In all patients a decrease in total Mayo score was detected (mean: 6.5 and 3.6 at screening and week 7, respectively; $p < 0.001$). Fecal calprotectin levels significantly decreased during the treatment phase (baseline: mean 778ug/g, range 192-1790ug/g; end of treatment: mean 305ug/g, range <30-1586ug/g; p=0.049), including 4 patients achieving undetectable levels at end of treatment. A decrease in endoscopic (Mayo score) and histologic (Riley index) indices of severity confirmed the beneficial effect. However, an increase of calprotectin levels and disease activity was observed after cessation of bilberry intake. No serious adverse events were observed. Our study was the first report on the promising therapeutic potential of a standardized AC-rich bilberry preparation in UC in humans. These results clearly indicate a therapeutic potential of bilberries in UC.

Although these results are highly promising, they clearly need to be confirmed in a larger controlled and randomized trial. We believe that ACs derived from Bilberries may provide an important and effective adjunctive treatment option in UC with very few - if any at all - side effects.

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Berry Compositional Chemistry and Biological Effects

Navindra Seeram, Ph. D.

Session Chair
University of Rhode Island

Current Research Review

Navindra P. Seeram, Ph.D., 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 Assistant Adjunct Professor in the UCLA School of Medicine. His research group, the Bioactive Botanical Research Laboratory, investigates plant foods and natural products for preventive and therapeutic effects against chronic human diseases. Dr. Seeram has co-authored over 121 original peer-reviewed research articles, 7 review articles, 16 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 based on Web of Science indexed citations from 2002-2012) 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.

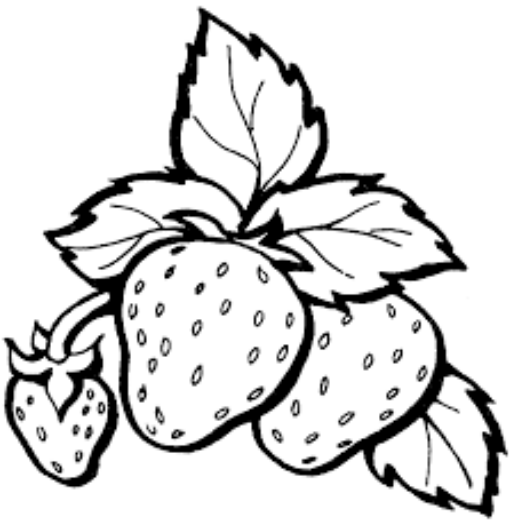
Chris Kruger, Ph.D.

University of Wisconsin-Madison

The Modernized Analytic Toolbox for Authentication, Standardization and Efficacy Evaluation of Natural Products

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.



The Modernized Analytic Toolbox for Authentication, Standardization and Efficacy Evaluation of Natural Products

Complete Phytochemical Solutions, LLC identifies authentication, standardization and efficacy as the most important criteria related to successful development, production and marketing of natural products. The complexity of nature creates a serious challenge to understanding how consumption of natural products, such as berries, results in beneficial health outcomes. To address this complexity, academics institutes and contract research organizations have made major advancements in the development and deployment of new analytic tools. Our efforts are currently directed at educating the natural product market place on how these analytic tools can be used to support authentication of raw and processed natural products, standardization of line processes for consistency in production, and efficacy evaluation as it relates to health benefits.

Macroscopic examination (sight, taste and smell) of a fresh or frozen berry is typically sufficient to assure the end consumer of the quality and authenticity of the end product. However, authentication becomes more difficult once a berry is subjected to processes such as juicing, pureeing or drying. At this stage the berry products still retain a portion of their sensory attributes but these observations alone are not sufficient for either qualitative (what does it look like) or quantitative (how much is there) analysis. To add further complication, utilization and further processing of berry co-products such as seeds, oils and pumice is a rapidly growing segment of the nutritional supplement and natural products market place. By the time these co-products enter the market place, often in the form of a power or encapsulated tablet, there is little or no sensory attribute that would allow a consumer to determine authenticity.

Fortunately, there are many advanced analytic tools available to assist natural product marketers and health researchers address issues of authentication, standardization and efficacy evaluation. Using cranberries as a case study, such a toolbox might consist of: the 4-(dimethylamino) cinnamaldehyde (DMAC) analysis for quantification of soluble proanthocyanidins (PAC), the butanol-HCl assay for quantification of insoluble PAC, high performance liquid chromatography (HPLC) for the identification and quantification of flavonols, anthocyanins and hydroxycinnamic acids, and mass spectrometry-based technologies such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) for the authentication of specific PAC structural features such as A-type interflavan bonds.

Natural product processors and marketers are fighting a continual battle in their efforts to relate the complexity of the phytochemical composition of their unique products to beneficial health related outcomes. Through panel discussions, webinars, scientific presentations and one-on-one consultation, experts in the field of phytochemistry are available to educate sellers and buyers on the tools available and the appropriate questions to ask in regards to product integrity. Authenticity, standardization and efficacy – are inseparable. Ingredient providers, formulators, marketers and health researchers can't address one issue without the other two. Consumers expect manufacturers to deliver on the quantity and quality of an ingredient necessary for the health outcome they're expecting.

Maurizio Battino, Ph.D.

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The effects of strawberry bioactive compounds on human health: a possible clue on the molecular mechanisms involved in the prevention of different chronic diseases

Maurizio Battino, PhD, Associate Professor of Biochemistry in the Dept of Clinical Sciences, Faculty of Medicine, Università Politecnica delle Marche (Italy) is the Director of the Centre for Health and Nutrition, Universidad Europea del Atlantico (Santander, Spain) and Director of Nutrition & Health projects and Master courses at FUNIBER on-line platform (Barcelona-Spain). His research group, the Bioenergetics Group, investigates the way of mitigating disease processes through the correct use of specific foods (mainly berries and dietary fats) and of their bioactive compounds. Targeted diseases are those related directly with mitochondrial impairment (e.g., fibromyalgia) and/or inflammation processes and oxidative stress including metabolic syndrome, cancer, atherosclerosis and periodontal diseases.

Dr. Battino has more than 25 years of experience in bioenergetics and in food research with special emphasis on the role of natural antioxidants and his studies are documented in more than 200 peer-reviewed research articles with h-index = 42 according to Google Scholar MyCitations or h-index = 36 according to Scopus and ISI Web of Science; he has also co-edited several books and special issues. He was BSc in Bologna, PhD in Catania and post-doc in Granada (Spain); he obtained a MS in International Communication Technology in Medicine (Ancona) and was awarded with a Doctor Honoris Causa degree by the University of Medicine and Pharmacy "Carol Davila" Bucharest (Romania). He currently reviews scientific articles for over three dozen peer-reviewed journals, serves as Editor-in-Chief of Journal of Berry Research (IOS Press), Diseases (MDPI) and Non Invasive Biomedicine (PHS), as Associate Editor of Molecules (MDPI), as Managing Editor of Mediterranean Journal of Nutrition & Metabolism (IOS Press) and in the editorial board of Food Chemistry (Elsevier), Plant Food for Human Nutrition (Springer), Nutrition and Aging (IOS Press), Antioxidants (MDPI).

The effects of strawberry bioactive compounds on human health: a possible clue on the molecular mechanisms involved in different chronic diseases

Epidemiological studies have already established a close association between a fruit/vegetable-rich diet and a significantly reduced incidence of chronic diseases (i.e., diabetes, cardiovascular diseases, cancers, etc.). Bioactive phytochemicals are therefore of increasing interest for their roles both in preventive strategies and as adjuvants in the treatment of the above pathologies. Among edible fruits, strawberries represent healthy foods since they contain many important dietary components including vitamins and minerals, and are a rich source of phytochemical compounds (Battino M. et al., 2009; Giampieri F. et al., 2012a). In the last 10 years, our research group studied the effective protection of strawberry extracts or strawberry consumption against oxidative damage on several models: (i) *in vitro* on human dermal fibroblasts (HDF), stressed through the exposure to UV-A radiation or chemical substances (Giampieri F. et al, 2012b; Giampieri F. et al., 2014a; Giampieri F. et al., 2014b), (ii) *in vivo* on animals, stressed with ethanol administration (Alvarez-Suarez J.M. et al., 2011) or Doxorubicin injection (Diamanti J. et al., 2014), and (iii) on healthy humans (Tulipani S. et al., 2008, 2009, 2011, 2014; Alvarez-Suarez J.M. et al., 2014). We found that strawberry bioactive compounds are able to protect HDF *in vitro*, counteracting the intracellular ROS production and resulting effective in an increase of cell viability, in a reduction of oxidative damage on membrane lipid and DNA and in an improvement of mitochondrial functionality. With regard to the *in vivo* studies, rats stressed with ethanol administration and fed with strawberry showed an increase in the antioxidant enzyme activities (SOD and catalase), a decrease in gastric lipid peroxidation and a concomitantly inhibition of the development of ethanol-induced gastric lesions. Similar beneficial effects were found in rats stressed with Doxorubicin injection. In human healthy volunteers, acute and medium-term strawberry intake led to significant increases in plasma total antioxidant capacity and in folate and vitamin C serum concentrations as well as to significant improvements of plasma lipid profile and erythrocyte and lymphocyte resistance to *ex vivo* induced oxidative damage.

In order to deepen and highlight the molecular mechanisms involved in the positive effects elicited by strawberry polyphenols, we are currently working on different experimental models. In RAW 264.7 macrophages stressed with LPS we are testing the anti-inflammatory effects of strawberry extract and preliminary results showed a reduction in ROS and NO production and lipid and protein oxidation as well as a restoration of antioxidant enzymes and mitochondrial functionality through AMP-activated protein kinase (AMPK) pathway modulation. At the same time, the reduction in gene expression of some inflammatory-cytokines, as IL-1β, IL-6 and TNF-α and in their related pathways as NF-kB and iNOS have been confirmed. We obtained similar results *in vivo* on rats supplemented with strawberry diet and subjected to LPS injection, confirming the protective role of strawberries against inflammation also *in vivo.*

In addition, in order to elucidate the molecular mechanisms involved in the effect of strawberry on lipid metabolism, we are currently working on three experimental models in which HepG2, HUVEC and 3T3-L1 cells are treated with two well characterized strawberry extracts (dried methanolic extract and anthocyanin fraction). The first results indicated that strawberry extracts beneficially influence the lipid profile by reducing lipid accumulation, low-density lipoprotein cholesterol, triglycerides levels and lipid peroxidation. These effects are mediated by the LKB1/p-AMPK stimulation with the consequent inhibition of the long chain fatty acids and cholesterol synthesis through the stimulation of the inactive form of the ACC and the inhibition of the HMGCR, respectively. Strawberry treatment also decreases intracellular ROS production and stimulates SOD and CAT activities.

Finally, it is known that polyphenols display other interesting effects, like pro-apoptotic or pro-oxidant properties, that in hyperproliferative cells can assume positive effects. In this context, we are currently analyzing the cytotoxic effects of strawberry extracts on uterine leiomyomas, one of the most common benign tumor of the uterus, and on breast cancer cells. Preliminary results showed that strawberry extracts present important toxic effects, leading to high death rate and intracellular ROS increase, as well as to the impairment of glycolysis and mitochondrial functionality; all these effects can be ascribed to a direct downregulation of the expression of p-AMPK, LKB1, NRF2, PGC-1α and SIRT1 and SIRT3.

Keywords: strawberry bioactive compounds, oxidative stress, aging, inflammation, lipid metabolism, cancer, gene modulation.

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Liewei Gu, Ph.D.

University of Florida

Bioavailability and bioactivity of phytochemicals in cranberries

Dr. Liwei Gu is an associate professor in the Food Science and Human Nutrition Department at University of Florida. He earned his PhD in Food Science and finished postdoc training at Arkansas Children's Nutrition Center. He was a research assistant professor at University of Arkansas for Medical Sciences before moving to University of Florida in 2008.

Dr. Gu's research programs focus on chemistry, bioavailability, and bioactivity of phytonutrients in berries and grapes. He published 60+ original researches on food science, nutritional science, and pharmaceutics journals. He directed 10+ research projects funded by governments and food industries. He was a recipient of Outstanding New Faculty Research Award at University of Florida in 2010. He was recognized as a Highly Cited Researcher by Thomson Reuters in 2014.

Bioavailability and Bioactivities of Phytochemicals in Cranberries

Cranberries and cranberry juice contain several types of phenolic phytochemicals. Among them, the A-type procyanidins received much attention because of their activity to inhibit the adhesion of uropathogenic bacteria. However, little was known about whether A-type procyanidins are absorbable in human intestine. A-type procyanidin oligomers were purified from cranberries and their transport was tested on differentiated human intestinal epithelial Caco-2 cell monolayers. Data indicated that procyanidin dimer A2, A-type trimers and tetramers traversed across Caco-2 cell monolayers with transport ratio of 0.6%, 0.4%, and 0.2%, respectively. This study suggested that A-type dimers, trimers, and tetramers were absorbable in human intestine after cranberry consumption albeit the absorption ratio will be low.

A major portion of ingested procyanidins is degraded by human microbiota in the colon into various phenolic compounds. These microbial metabolites are thought to contribute to the health benefits of procyanidins in vivo. (-)-Epicatechin, (+)-catechin, procyanidin B2, procyanidin A2, partially purified apple and cranberry procyanidins were incubated with human microbiota under an anaerobic condition. GC-MS analysis showed that common metabolites of all six substrates were benzoic acid, 2-phenylacetic acid, 3-phenylpropionic acid, 2-(3'-hydroxyphenyl)acetic acid, 2-(4'-hydroxyphenyl)acetic acid, 3-(3'-hydroxyphenyl)propionic acid, and hydroxyphenylvaleric acid. 5-(3',4'-Dihydroxyphenyl)-γ-valerolactones and 5-(3'-hydroxyphenyl)-γ-valerolactones were identified as the microbial metabolites of epicatechin, catechin, procyanidin B2, and apple procyanidins but not from the procyanidin A2 or cranberry procyanidin ferments. 2-(3',4'-Dihydroxyphenyl)acetic acid was only found in the fermented broth of procyanidin B2, A2, apple, and cranberry procyanidins. The mass recoveries of microbial metabolites range from 20.0 to 56.9% for the six substrates after 24 h of fermentation. This study showed that microbial metabolites of A-type procyanidins in human large intestine were different from those of monomers or B-type procyanidins.

Cranberry juice is known to prevent urinary tract infections whereas apple juice does not. A 1H-NMR based metabolomic approach was used to reveal global metabolic changes caused by cranberry juice consumption. Eighteen female college students were recruited and given either cranberry or apple juice for three days using a cross-over design. Plasma and urine samples were collected and analyzed using 1H NMR followed by multivariate analyses. No metabolic difference was observed in plasma before and after juice consumption. However, metabolome in plasma and urine after cranberry juice consumption were different from those after apple juice consumption. Cranberry juice consumption caused a greater increase in urinary excretion of hippuric acid and a higher level of citric acid in the plasma. Furthermore, cranberry juice decreased the plasma level of lactate, D-glucose, and two unidentified metabolites compared to apple juice consumption. Taken together, our studies showed that A-type procyanidins in cranberries were absorbed and metabolized differently compared with B-type ones. Cranberry juice consumption in women caused detectable metabolomic changes in blood and urines. All these may help to explain the health benefits of cranberry consumption, especially its ability to prevent urinary tract infection. This research was supported in part by Ocean Spray Cranberries Inc.

David Rowley, Ph.D.

University of Rhode Island

Cranberry oligosaccharides decrease biofilm formation by uropathogenic *Escherichia coli*

Professor of Biomedical and Pharmaceutical Sciences, Alex & Ani Positive Impact Laboratory, College of Pharmacy, University of Rhode Island

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 oligosaccharides decrease biofilm formation by uropathogenic *Escherichia coli*

Urinary tract infections (UTI) commonly occur in the kidney and bladder. UTI patients often experience frequent recurrence and increasing susceptibility to the drug resistant uropathogens (1). Over 80% of the UTIs are associated with Escherichia coli, which can form biofilms on the bladder wall that provide protection against antibiotic treatment (2, 3). Evidence suggests that consumption of cranberry juice can decrease the presence of bacteria in the urine and reduce UTI symptoms (4). Phenolic compounds from cranberry have been extensively studied for their antimicrobial properties (5-7). However, the carbohydrate constituents from cranberry have received far less attention (8, 9). In this investigation, we purified phenolic-free carbohydrate constituents from cranberry (Vaccinium macrocarpon) and characterized their structures using mass spectrometry and nuclear magnetic spectroscopy (NMR). Our results revealed constituents consistent with oligosaccharides possessing various degrees of polymerization. Further structural analysis revealed that these constituents are mainly composed of xyloglucan and arabinan oligosaccharides. In antimicrobial assays, one enriched fraction reduced biofilm production by the uropathogenic Escherichia coli CFT073 by over 50% at 1.25 mg/mL with no adverse effects on bacterial growth. These results suggest that, in addition to phenolic compounds, oligosaccharide components of cranberry products should be further explored for their role in the prevention of urinary tract infections.

Key Words: Cranberry, Oligosaccharide, Urinary Tract Infection, E. coli, biofilm

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Berries and Metabolism

Ron Prior, Ph. D.

Session Chair
University of Arkansas

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.

Colin Kay, Ph.D.

University of East Anglia

Newly discovered bioactive metabolites of berry anthocyanins: potential health effects and implications for future research

Current research involves the investigation of phytochemical metabolism and bioactivity following human feeding interventions and in cultured vascular and inflammatory cells. Current Lab group includes 3 PhD and 1 MSc student. Research group is located in the Metabolite & Biomarker Analytics Suite in the Norwich Medical School and houses high performance liquid chromatographers and mass spectrometers and accompanying clinical and cell culture facilities. Core funding is supported primarily by the Biotechnology and Biological Sciences Research Council (BBSRC).

Selected Recent Publications

1. Phenolic metabolites of anthocyanins modulate mechanisms of endothelial function. Michael Edwards, Charles Czank, Gary M. Woodward, Aedín Cassidy, Colin D. Kay. In: Journal of Agricultural and Food Chemistry, 2015 (in press; DOI 10.1021/jf5041993).
2. Methods for isolating, identifying and quantifying anthocyanin metabolites in clinical samples. de Ferrars, Rachel M; Czank, Charles; Saha, Shikha; Needs, Paul W; Zhang, Qingzhi; Raheem, K Saki; Kroon, Paul A; Kay, Colin D. In: Analytical Chemistry, Vol. 86, No. 20, 21.10.2014, p. 10052–10058.
3. The pharmacokinetics of anthocyanins and their metabolites in humans. De Ferrars, R M; Czank, C; Zhang, Q; Botting, N P; Kroon, P A; Cassidy, A; Kay, C D. In: British Journal of Pharmacology, Vol. 171, No. 13, 01.07.2014, p. 3268-3282.
4. Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. de Ferrars, Rachel M; Cassidy, Aedín; Curtis, Peter; Kay, Colin D. In: Molecular Nutrition & Food Research, Vol. 58, No. 3, 01.03.2014, p. 490-502.
5. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a 13C-tracer study. Czank, Charles; Cassidy, Aedin; Zhang, Q.; Morrison, Derek; Preston, T.; Kroon, Paul; Botting, N. P.; Kay, Colin. In: American Journal of Clinical Nutrition, Vol. 97, No. 5, 05.2013, p. 995-1003.

Newly discovered bioactive metabolites of berry anthocyanins: *potential health effects and implications for future research*

Berries contain an extensive array of bioactive components, including phytochemicals, fibre, vitamins and minerals. Anthocyanins, found in the skins of berries, are often associated with health benefits [1], predominantly related to cardio-metabolic activity [2-4]. However, anthocyanins are prone to extensive metabolism and the impact this has on their biological activity is presently unknown. After consumption, anthocyanins undergo significant biotransformation as a result of gastric pH, enzymatic and microbial activity. Consequently, most studies report less than 1% recovery of ingested parent/precursor anthocyanins [1, 5, 6]. Until recently, the fate of the remaining 99% of ingested anthocyanins was unknown. We recently characterised the bioavailability of a labelled anthocyanin (13C5-cyanidin-3-glucoside) in 8 male participants [7, 8]. Serum, urine, feces and breath were collected over 48 h and analysed using isotope-ratio mass spectrometry and liquid chromatography-tandem mass spectrometry (Q-TRAP). 44% of the 13C label was recovered, with a calculated relative bioavailability of 12% [7]. Greater than 30 unique metabolites were identified [8], including phloroglucinaldehyde, phenolic, hippuric, phenylacetic and phenylpropenoic acids. Maximal plasma concentrations ranged between 0.01 and 2 μM. These results indicated that anthocyanins were extensively metabolised in vivo. Therefore, we hypothesised that the bioactivity of anthocyanins results from circulating phenolic metabolites.

We explored the activity of the metabolites at physiologically relevant concentrations (0.1-10 μM) in several cell culture models (in HUVEC, AVSMC & THP-1 cells). Here, we investigated the activity of cyanidin-3-glucoside relative to 4-hydroxybenzoic acid, benzoic acid-4-glucuronide, benzoic acid-4-sulfate, phloroglucinaldehyde, 3,4-dihydroxybenzaldehyde, protocatechuic acid, homoprotocatechuic acid, protocatechuic-3-glucuronide, protocatechuic-4-glucuronide, protocatechuic-3-sulfate, protocatechuic-4-sulfate, vanillic acid, vanillic acid-4-glucuronide, vanillic acid-4-sulfate, isovanillic acid, isovanillic acid-3-glucuronide, isovanillic acid-3-sulfate, homovanillic acid, vanillin, and ferulic acid. Phenolic glucuronide and sulfate conjugates were synthesised where commercial standards were not available. Activity was assessed across 20 different screening assays, including: Cytochrome c reduction, nitric oxide and superoxide production, expression of eNOS, NOX, p22phox, p47phox, endothelin-1, HO-1, IL-1B, oxLDL-stimulated IL-6, CD40L-stimulated IL-6, VCAM-1, TNF induced VCAM and IL6, LPS induced TNF, tissue factor, Nrf2 and NF-κB pathway inhibition. Here the parent/unmetabolised anthocyanin was, in most cases less active than its phenolic metabolites, with the exception of eNOS activity [9-11]. No metabolites altered the expression of endothelin-1 or nitric oxide, 3 metabolites induced eNOS, 2 metabolites reduced angiotensin-II induced superoxide, no metabolites induced NOX or p47phox, 1 metabolite upregulated p22phox and 3 metabolites induced HO-1. In vascular inflammation and adhesion screens 10, 9 and 6 of the metabolites reduced IL-6 following CD40L, oxLDL or TNF-α stimulation (respectively), while 10 and 6 of the metabolites reduced sVCAM-1 following CD40L or TNF-α stimulation (respectively). 2 metabolites attenuated IL-1β-stimulated phosphorylation of NF-κB p65 and 1 metabolite induced Nrf2. In monocyte activity assays, 5 metabolites reduced LPS-induced TNF-α, 1 metabolite induced IL-1β, and no metabolites were active on HO-1, tissue factor or NFκB.

Overall the evidence indicated that the bioactivity of anthocyanins in vivo likely results from a low exposure to a variety of structurally similar metabolites modifying various inflammation and cellular adhesion pathways. Future research should focus on the mechanisms of action of phenolic metabolites of anthocyanins.

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Ana Rodriquez Mateos, Ph.D.

University of Reading

Berries and cardiovascular health:
is it really the anthocyanins

Dr. Rodriguez-Mateos is a Research Group Leader at the Division of Cardiology, Pulmunology and Vascular Medicine of the University of Dusseldorf, Germany.

She received her PhD from the University of Reading, UK. She conducted her postdoctoral studies at the Department of Food and Nutritional Sciences of the University of Reading, where she began to investigate the bioavailability of dietary phytochemicals and their impact on vascular function. She has been a visiting researcher at universities in Spain, Brazil, Japan and US. Her current research interests are to elucidate the role of dietary polyphenols on cardiovascular health, to investigate the factors affecting the bioavailability and bioactivity of dietary polyphenols, and to investigate their mechanisms of action in the vascular system. She is particularly interested in investigating the potential health benefits of anthocyanin-rich foods such as berries in humans.

Berries and cardiovascular health: is it really the anthocyanins ?

Recent epidemiological and human intervention studies suggest that berry consumption may have cardiovascular health benefits1. Berries are rich sources of potential bioactive compounds such as (poly)phenols, fiber, minerals and vitamins. The most abundant (poly)phenolic compounds in berries are anthocyanins, flavan-3-ols, flavonols and phenolic acids. In the last few years, several clinical trials have been conducted investigating the effect of berry consumption on clinically relevant biomarkers of cardiovascular disease (CVD) risk, such as blood pressure, endothelial function, arterial stiffness and blood lipids, with mixed results1. The small amount of existing studies and the wide heterogeneity between them as for the levels of intake, study population, and length, may be the reason for the inconsistencies found in the available data. Whether berry consumption can improve vascular function and health when given over relevant time periods and in relevant amounts is currently not known. This may also be due to an incomplete understanding of the intake and time-dependence of vascular effects. The "negative" results observed in many of the randomized controlled trials (RCTs) performed so far may be because the time-point of maximal effects might have been missed or amounts/doses too high or too low. Another important limitation is that the bioavailability of berry (poly)phenols have not been assessed in most of the RCTs conducted up to now. It is an important prerequisite to assess whether (poly)phenols, or more likely their metabolites, are indeed entering the circulatory system and may only then qualify for mediating the observed effects. The basic understanding of berry (poly)phenol absorption, metabolism and excretion is incomplete and this needs to be addressed in order to understand their potential health benefits on the vascular system.

We have conducted several randomized controlled trials aiming to investigate the time-course and the intake-dependence of berry (poly)phenol-related improvements in vascular function in healthy individuals. The potential role of berry (poly)phenols in the modulation of vascular function was investigated by monitoring changes in vascular function together with changes in the major (poly)phenol derivatives/metabolites in plasma and urine.

In order to confirm whether the (poly)phenols are the major bioactives responsible for the effects observed we have investigated the effects of fiber, minerals, vitamins and pure anthocyanins on vascular function. Our results suggest that anthocyanins are major contributors to the beneficial effects of berries on vascular function, however other (poly)phenols and bioactives in berries may have a role and act synergistically enhancing the long term effects of berries on cardiovascular health.

Keywords : berries, (poly)phenols, vascular function, cardiovascular health, bioavailability, clinical trials

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Daniele Del Rio, Ph.D.

University of Parma

Raspberry ellagitannin metabolites in experimental models of chronic disease

Daniele Del Rio is Associate Professor of Human Nutrition at the University of Parma. He is running the Laboratory of Phytochemicals in Physiology at the Department of Food Science and is the co-founder of the LS9 "Bioactives & Health" Interlaboratory Group, where the biological activity of human and microbiota derived phytochemical metabolites represents one of the core research topics. Daniele is an Honorary Visiting Scholar at the UK Medical Research Council Human Nutrition Research Unit in Cambridge, a Visiting Fellow of the Wolfson College, University of Cambridge and a senior collaborator of the Need for Nutrition Education/Innovation Programme (NNEdPro), an independent knowledge generation and research platform overseen by the British Dietetic Association. Dan is also the Commissioned Reviews Editor of the Journal of Human Nutrition and Dietetics (the official Journal of the British Dietetics Association) and Associate Editor of the International Journal of Food Sciences and Nutrition.

He is a member of the Board of Directors of the University Spin-Off "Madegus", focused on Nutritional Education for Children.

Daniele has been recently listed among the Thomson-Reuters Highly Cited Researchers. His publications could be retrieved at Google Scholar and Researcher ID

Raspberry ellagitannin metabolites in experimental models of chronic disease

Ellagitannins are a complex subclass of hydrolysable tannins present mainly in berries (raspberries in particular), pomegranate, walnuts, and oak-aged red wines that have shown to exert different bioactivities (Zanotti et al., 2014). They are catabolized by the colonic microbiota to form a series of urolithins possessing a 6H-dibenzo[b,d]pyran-6-one structure with different phenolic hydroxylation patterns (Fig. 1) (Sala et al 2015).

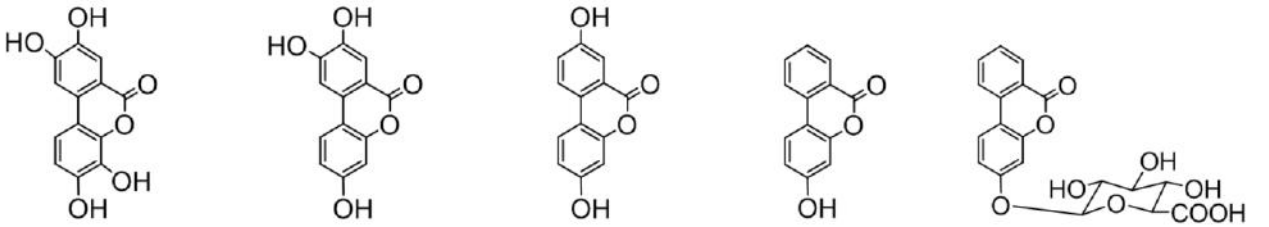

Fig.1: Urolithins are 6H-dibenzo[b,d]pyran-6-one structures with different phenolic hydroxylation patterns.

After absorption from the large intestine, urolithins appear in circulation as glucuronide, sulphate and methylated metabolites at concentrations ranging between high nM and low μM, and they can also accumulate in certain tissues, (García-Muñoz, Hernández, Pérez, & Vaillant, 2014). These human/microbial metabolites of ellagitannins have been shown to exert several different specific positive actions in many contexts of human chronic disease onset, with a peculiar focus on the inflammatory process.

The aim of this talk will be highlighting the stability and bioactivity of urolithins A, B, C, and D (Uro A, Uro B, Uro C, and Uro D) as well as a urolithin B-3-O-glucuronide (Uro B-gluc) in the framework of several experimental models of chronic diseases, describing their interaction with various pathogenic mechanisms typical of diabetic cardiomyopathy, atherogenesis, endothelial function, certain types of cancer, and specific neurodegenerative diseases.

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Emilie Fromentin, Ph.D.

NATUREX-DBS

Aronia an up and coming healthy berry

Dr. Emilie Fromentin has received her Ph.D. from AgroParisTech (France) in analytical chemistry and pharmacology in 2010, in collaboration with her laboratory at Emory University School of Medicine (Atlanta, USA). She is currently research and development manager at NATUREX-DBS, a company specialized in the manufacturing of standardized, polyphenol-rich nutraceutical ingredients and extracts from North American berries. She manages the research science platform to develop scientifically substantiated, chemically characterized ingredients from cranberry, blueberry and chokeberry. Her research is primarily focused on risk factors and conditions that affect the aging population, with an emphasis on cardiovascular health, cognitive performance and urinary tract health.

Aronia an up and coming healthy berry

Aronia melanocarpa (Chokeberry) is native to North America and its rangeland extends from the northeastern part of North America and the Great Lakes area to the higher part of the Appalachians in the south, where it occurs in mountain bogs and balds. Aronia berries are consumed fresh, juiced, or processed further for jams, juice blends, extracts, or food colorants. Among the compounds of interest, chokeberries are particularly rich in proanthocyanidins, flavanols, anthocyanins, flavonoids (particularly quercetin derivatives), chlorogenic acids, neo-chlorogenic acids, caffeic acid, triterpenes (isolated from the seeds), and fibers.1

Powdered aronia extracts rich in polyphenols show strong antioxidant capacity and have been further investigated in preclinical and clinical studies. The clinical studies conducted so far have suggested that aronia may play a role in the maintenance of cardiovascular functions. This effect might be exerted via modulation of inflammatory cytokines and biomarkers, reduction of platelet aggregation and/or improvement of endothelial functions.2 In pilot clinical studies, significant improvement of systolic blood pressure, diastolic blood pressure, serum endothelin (ET-1), serum total cholesterol, serum low density lipoprotein and serum triglycerides were observed after a 2 months intake of chokeberry extract containing 45 mg of anthocyanins.3 In another study, the reduction in SBP and DBP was correlated with a reduction in the activity of angiotensin converting enzyme (ACE) activity.4

The modulation of inflammatory markers was essentially demonstrated in vitro, chokeberry concentrate inhibited the release of proinflammatory cytokines in human peripheral monocytes and the activated the NF-κB pathway in macrophages;5 and chokeberry extract inhibited the expression of ICAM-1 and VCAM-1 in human aortic endothelial cells.6 In humans, an aronia extract significantly reduced key markers of endothelial inflammation, including oxidative stress biomarkers and increased adiponectin levels.4

In animal models of prediabetes, modulation of insulin and hypoglycemic effects were observed and may suggest a potential beneficial effect on glycaemia that still remains to be demonstrated in humans.7 More work remains to be done to understand the potential effect of aronia on metabolic syndrome in humans.

In conclusion, aronia ingredients, owing to their rich phytochemical composition and antioxidant properties may exert very promising health benefits in areas that might be much broader than those already studied, with possibly enhancement of vascular function and inflammation as a key central mechanism for potential beneficial effects on health that may include improvement of vision, cognitive function, reduction of adiposity, improvement of microcirculation, cellulitis, cold condition…

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Poster Presentation Abstracts

Isolated procyanidins modulate secretion of airway inflammation chemokines CCL11 and CCL26 in human alveolar epithelial cells

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Epidemiological evidence suggests populations that consume diets rich in procyanidins, polyphenolic secondary plant metabolites, are less susceptible to inflammatory diseases, with the major source of dietary dimeric procyanidins being fruits. Asthma is an inflammatory lung disease with an estimated 100 million affected individuals worldwide. New Zealand has the world's second highest rate of asthma. One of the central characteristics of asthma is the infiltration of eosinophil cells and other immune cells into the lung. The chemokine eotaxin, found in three isoforms CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL26 (eotaxin-3), is one biomarker known to be over-expressed in asthma, and causes eosinophil cell migration into lung tissue. The aim of the present study was to evaluate isolated fruit procyanidins as a means of modulating the inappropriate inflammatory response present during asthma. Using a human alveolar epithelial cell line (A549) model, we have demonstrated that a particular procyanidin (at a physiologically relevant concentration) inhibited both CCL11 and CCL26 secretion when incubated (6 h) prior to an inflammatory challenge. Conversely, another distinct procyanidin inhibited CCL11 secretion only. These data suggest that the mechanism of inflammation management by these two procyanidin compounds may be via the modulation of different signalling pathways or divergent physical interactions. Understanding in more detail the role of fruit procyanidins in managing inflammation, and the mechanisms by which this could occur would allow for the potential use of single and/or multiple procyanidins in fresh and processed foods as natural means to prevent and manage inflammatory illness, limiting the use of pharmaceutical interventions, and assisting with improving human health.

Phenolic compounds in 30 genotypes of red raspberry (*Rubus idaeus* L.)

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Raspberries contain high concentrations of phenolic compounds, especially anthocyanins and ellagitannins. Phenolic compounds contribute both to color (anthocyanins) and the observed health effects of eating raspberries. Several factors will influence concentration of phenolic compounds in the berries. These factors are genotype, growing conditions, maturity and processing, with genotype as the most fundamental cause of variation.

The aim of the study was to provide an overview of content and variation of phenolic compounds in 30 raspberry cultivars grown in Norway, to be able to evaluate their visual attractiveness (color) and color stability, as well as potential health effects. The effect of ripening stage (light red, red, dark red) on phenolic composition was determined for one genotype.

The most abundant phenolic compounds in the ripe berries were anthocyanins and ellagitannins with total concentrations varying from 40 – 145 mg/100 g fw and 57 – 151 mg/100 g fw, respectively. The concentrations of flavonols and ellagic acid glycosides were only about 1 mg/100 g fw. The anthocyanin profiles were dependent on genotype. Cyanidin-3-sophoroside was the most abundant anthocyanin in most samples, contributing 32-78% of anthocyanin content in raspberries. In some genotypes cyanidin-3-(2^{G}-glucosylrutinoside) was the dominating anthocyanin and these genotypes were clustered characterized by high concentration of all rutinosides (of cyanidin and pelargonidin), while these compounds were not present at all in other genotypes. Ripening stage, within the range suitable for consumption, did not considerably affect phenolic profile of berries of ‘Glen Ample’. The least mature berries of ‘Glen Ample’ contained higher concentrations of ellagitannins and lower concentrations of anthocyanins than the more-ripe berries.

Environmental and genetic effects on the content of polyphenols in cloudberry (*Rubus chamaemorus*)

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Cloudberry (*Rubus chamaemorus*) is a dioecious perennial rhizomatous plant native to arctic regions. Cloudberries contain several polyphenols. Polyphenols are linked to prevention of age-related diseases like cancer and cardiovascular diseases. There are several groups of polyphenols with various bioactive effects. Ellagitannins, the most abundant phenolic compound in cloudberry, are a group of polyphenols recognized for its nutritional and pharmacological potential both as an antioxidant as well and an antiviral agent.
It is widely known that genetics greatly influence the content of chemical constituents in berry fruits. In addition to genetics, varying environmental conditions, like weather within and between seasons are also affecting the contents of bioactive compounds. In our experiment, four different clones of cloudberry have been grown in outdoor plots. The plots were established in 2010 and have been harvested at different times throughout the harvest seasons of 2012, 2013 and 2014. Climatic effects within and between seasons, effects of genotype as well as interactions between genotype and climate have been evaluated for contents of total anthocyanins, total phenols and ellagic acid.

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Effect of strawberry consumption timing relative to meal intake on the pharmacokinetic parameters and bioavailability of strawberry anthocyanins

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Strawberries are a rich source of polyphenols, especially anthocyanins. Anthocyanins impart red color to strawberries, but are also associated with health promoting effects for chronic disease prevention. However, if the health promoting action of strawberries or their anthocyanins is dependent on bioavailability and or achieving a certain blood concentration or exposure time to impart biological activity then understanding the factors that influence these variables are critical. Therefore, the present study was conducted to determine the effect of timing of strawberry consumption relative to meal on the pharmacokinetic parameters and bioavailability of strawberry anthocyanins. A randomized, 3-arm, placebo controlled crossover trial was conducted on overweight (BMI: 26 ± 2 kg/m2) healthy adults (n = 14). Subjects came on 3 different occasions for 10 hours and received 3 study drinks on each visit: two placebo drinks and one strawberry drink made from 12g freeze dried powder. The strawberry drink was given at either 2 hours before the breakfast meal (time = 0h), with the meal (time = 2h) or after the meal (time = 4h) and the placebo drinks were given at the alternative time points depending on strawberry drink assignment. Plasma samples were collected from time 0 to 10 h with one hour interval in between and analyzed for anthocyanin metabolites using QQQ LC-MS. Five different anthocyanin metabolites (cyanidin-3-glucoside, C3G; cyanidin-3-rutinoside, C3R; pelargonidin-3-glucoside, P3G; pelargonidin-3-rutinoside, P3R and pelargonidin glucuronide, PG) were identified in plasma. PG was the major metabolite with Cmax (maximum concentration of metabolite in time period of 0-10h) of 38.01±6.64, 12.79±2.11, 34.47±7.32 nmol/L when strawberry beverage was consumed before the meal, with the meal and after the meal, respectively. Tmax (time point when maximum concentration is achieved) of PG was achieved at 1.7±0.2, 4.8±0.3, 5.9±0.3 h when strawberry beverage was consumed before the meal, with the meal and after the meal, respectively. There was a significant ($p<0.05$) increase in the Cmax and AUC (area under curve) of PG when the strawberry beverage was consumed before the meal, compared to consumption with or after the meal. The bioavailability of pelargonidin anthocyanins as assessed by PG was significantly higher ($p<0.05$) when the strawberry beverage was consumed before or after the meal compared to with the meal. Our results give strong indication that timing of strawberry consumption relative to a meal impacts anthocyanin bioavailability and pharmacokinetic variables that may be important for delivering their maximal health benefits.

Aronia berry consumption modulates colonic IL-10 and inhibits wasting in a T cell adoptive transfer model of colitis

Derek Martin and Bradley W. Bolling

Aronia berries are rich in anthocyanin and proanthocyanidin polyphenols. Berry components or their metabolites may modulate immune function and protect against chronic inflammation. The objectives of this study were to evaluate the effect of aronia consumption on colonic cytokines and colitis induced by adoptive transfer of naïve T-cells to immunocompromised mice. C57/BL6 mice or recombinase activating gene (RAG)$^{-/-}$ mice were fed control diets or control diets supplemented with 4.5% lyophilized 'Viking' aronia berries. Colitis was induced in RAG$^{-/-}$ mice by injecting CD4^{+}CD45RB^{high} splenocytes flow sorted from wild type mice. Aronia consumption inhibited weight loss and improved survival after adoptive T-cell transfer to RAG$^{-/-}$ mice. In contrast, histopathological analysis of the colons indicated these mice had more mild to moderate colitis compared to controls, which may reflect disease progression in mice with longer survival. Colon tissues from C57/BL6 mice fed aronia or control diets were isolated and incubated in growth media to determine the extent aronia consumption modulates cytokine production. Colons from aronia-fed C57/BL6 mice had ~2-fold IL-10 production of mice fed control diets, however, IL-6, IL-17, and TNF-α were not significantly different between the groups. Lipopolysaccharide-stimulation of colon tissue did not reveal any further group differences in cytokine production. Therefore, aronia appears to prevent wasting induced by T-cell induced colitis and further work is needed to determine the capacity of aronia to modulate IL-10 at the initiation of colitis.

Therapeutic Effects of Various Solvent Extracts of Blue Honeysuckle Berry (*Lonicera caerulea L.*) Against Prostate Cancer Cells

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Prostate cancer (PCa) is the second most common cancer among men in the United Sates. It is estimated 1 in 5 men will be diagnosed with prostate cancer and approximately 220,800 new cases are predicted in 2015 alone. Common treatments for prostate cancer include hormonal therapy, chemotherapy, radiation and surgery. However, factors like patient health, drug resistance, specificity and toxicity can result in poor disease prognosis. Recent studies are being focused on natural products and their components for alternative therapeutics.

Blue Honeysuckle (Lonicera caerule L.) a berry native to northeast Asia, is known to be rich in Vitamin C and polyphenols such as anthocyanins, flavonoids and phenolic acids. Polyphenols are known to have several therapeutic effects such as anti-inflammatory, antioxidant and anti-carcinogenesis. Our current study aims to analyze sequential solvent extracts of Blue Honeysuckle (BHS) with Hexane, Ethyl Acetate, Methanol and Water respectively. These fractions were used to test therapeutic properties of BHS on DU 145 and PC-3 cancer cell lines. The goal was to identify most effective fraction for isolation of potential anti-cancer compounds.

Cell Viability Assays were used to identify most effective BHS fraction. DU 145 and PC-3 cancer cells were treated with different doses of BHS factions over various time periods (24, 48, 72 Hr). Results indicated that Hexane extract (HE) showed highest inhibition of cell viability in both cell lines in comparison to other BHS extracts. HE displayed consistent inhibition (≈ 55%) between 50-100 μg/mL doses in both cell lines (72 h for DU 145 and 48 h for PC-3). HE fraction showed to have an IC_{50} of ≈ 89.6 μg/mL for DU 145 cells at 72 h, and an IC_{50} of ≈ 117.4 μg/mL for PC-3 cells at 48 h. Moreover BHS extracts showed both a dose and time dependent effect on cell viability.

Western Blotting was performed to identify potential anti-cancer mechanism of BHS against prostate cancer cells. Both DU 145and PC-3 cell lines after 24 Hr treatment with 100 μg/mL of various BHS fractions showed modulation in apoptosis (Caspase 3,8,9 and AIF), autophagy (LC3 A/B) and cell proliferation markers (C-MYC, P-TEN). At same dose HE also exhibited inhibition of cell proliferation and disrupted the migration ability of metastatic prostate cancer cells *in vitro.* Additional studies with inhibitors for specific markers showed involvement of intricate apoptotic signaling pathways. Further analysis of these pathways is required to illustrate potential anti-cancer mechanisms seen in BHS treatments. In conclusion, results from our study warrant further evaluation of BHS berry for potential prostate cancer treatment.

to determine the capacity of aronia to modulate IL-10 at the initiation of colitis.

Relationship of Aronia berry polyphenol metabolism to its cholesterol-lowering activity in former smokers

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Abstract

Aronia berry extract is a rich source of anthocyanins and other polyphenols which may lower plasma cholesterol and reduce cardiovascular disease risk. We hypothesized that the cholesterol-lowering efficacy of aronia polyphenols was related to its metabolism and bioavailability. Former smokers were selected as a target population because of their higher risk of cardiovascular disease. A single-dose pharmacokinetic trial was used to evaluate the acute absorption, metabolism, and excretion of 500 mg encapsulated aronia berry extract in n = 6 adults. Anthocyanins were extensively metabolized and less bioavailable than colonic polyphenol catabolites. Levels of polyphenol metabolites in fasting plasma and urine correlated with total AUC in plasma and urine (r = 0.8602, and r =0.9660, respectively). This suggests that fasting blood and urine collections after supplemental aronia extract consumption could be used to estimate bioavailability and metabolism. A 12-week multiple-dose, double blind, and placebo-controlled trial was conducted in 49 adults (n = 24/placebo, n = 25/aronia) to evaluate the ability of 500 mg aronia extract/day to modulate plasma lipids. Aronia consumption reduced fasting plasma total cholesterol level by 8% during the 12-week treatment by repeated measures with or without confounder adjustment (P = 0.0130, and P = 0.0140, respectively). Also, individuals consuming aronia extract had a distinct polyphenol metabolite profiles compared to individuals consuming the placebo. Higher urinary cyanidin-3-*O*-galactoside, peonidin-3-O-galctoside, and 3-(4-hydroxyphenyl) propionic acid were associated with reduced plasma LDL. Therefore, bioavailability and metabolism appear to be associated with the cholesterol-lowering activity of aronia berry polyphenols.

Using untargeted metabolomics for profiling phytochemical changes in black raspberries due to thermal processing

Matthew D. Teegarden, Morgan J. Cichon, and Steven J. Schwartz

Clinical and laboratory studies have implicated black raspberries (BRBs) and their associated phytochemicals in the modulation of several chronic diseases including oral and esophageal cancers. While these effects are thought to be partially mediated by certain classes of berry phytochemicals such as anthocyanins and ellagitannins, the bioactivity of BRBs cannot be explained by these specific compounds alone. In addition, most research on the health benefits of BRBs is conducted using freeze-dried or otherwise minimally processed products, yet BRBs are typically consumed as thermally processed goods such as jams, jellies, and syrups. For this reason, a BRB nectar product was developed at the Ohio State University for use in human clinical trials. The objective of this work was to profile the chemical changes that result from thermal processing of BRBs to nectar as an essential step towards practical translation of clinical research. An untargeted LC/MS-based metabolomic approach was used to profile the polar compounds present in freeze-dried BRB powder and finished nectar. Of the over 5000 compounds detected, the processed and unprocessed products were found to differ significantly in hundreds of phytochemicals, including several compounds unique to the nectar. The global phytochemical profiles obtained through this work demonstrate the advantages of employing untargeted metabolomics and will further facilitate understanding of potential clinical outcomes attributed to the BRB nectar or similarly processed products.

Are the effects of blueberry anthocyanins on executive function in children mediated by cognitive demand?

Adrian R Whyte, Daniel J Lamport, Graham Shafer, Claire M Williams

Introduction and background: Previous research by our group indicates that a 235mg anthocyanin-rich blueberry drink can improve verbal memory and executive function in children aged 7-10 years, 1-6 hours post ingestion. Interestingly, the findings indicated that the sensitivity of the cognitive tests to detect anthocyanin-related changes increased with task difficulty. Specifically, on a modified attention network task (MANT), positive effects of anthocyanin consumption were found on cognitively demanding incongruent trials but not easier trials.

Aims: To investigate whether there is a direct association between cognitive demand and task performance following consumption of an anthocyanin-rich blueberry drink in children aged 7-10 years.

Design: Nineteen children (mean age 8 years) consumed a 253mg anthocyanin drink containing 30g freeze dried wild blueberry powder (108kcal)and a 13mg anthocyanin control drink matched for glucose, fructose and vitamin C following a counterbalanced, crossover, double blind design. Both drinks were prepared with a 30ml orange cordial and 170ml water. A seven day washout occurred between the two test days. Cognitive performance (with the MANT) was assessed at baseline and 3 hours following treatment. Twenty four hours prior to arrival the children consumed a low-flavonoid diet and a standardised lunch was provided prior to testing. Treatment drink ingestion was standardised for all test days at 13:00 hours

Cognitive Test: The MANT is based upon the Flanker task and requires the participant to respond by pressing one of two buttons according to whether a target stimulus is pointing left or right. Task difficult was manipulated in two ways; (i) by introducing a non-target stimulus, which was either congruent (easier) or incongruent (more difficult) with the target stimulus, and (ii) by increasing or decreasing the load such that a high load contained more stimuli on the screen. Thus, the most demanding trials were incongruent high load trials.

Results: The blueberry drink was associated with faster response times relative to the control drink as indicated by a significant main effect of Drink in the ANOVA model ($p<.05$). In support of the hypothesis, the benefits of the blueberry drink were greatest on more cognitive demanding high load trials.

Conclusions: This research provides further evidence that consumption of an anthocyanin-rich blueberry drink can provide acute cognitive benefits for children. Benefits were observed for executive function, which broadly speaking, encompasses attention, cognitive flexibility, goal setting, and information processing. More specifically, the present findings indicate the anthocyanin-rich drinks are particularly beneficial for more complex executive functions which require a high degree of attention and inhibition, as indicated by strongest effects being seen on the most demanding trials. The implications here are that blueberry drinks may be useful for enhancing attention and maximising performance in children throughout the day, which is particularly useful within an academic environment. We are currently investigating whether this cognitive demand effect is replicated in healthy young adults, the results of which we plan to include in our final poster.

Cranberry Proanthocyanidins Induce Autophagy via Modulation of Reactive Oxygen Species and Inactivation of PI3K/AKT/mTOR Signaling in Barrett's and Esophageal Adenocarcinoma Cells

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There is growing interest in utilizing food-based bioactive constituents for potential long-term administration to cohorts with elevated cancer risk. Specific favorable qualities of cranberries and cranberry derived extracts for cancer prevention include they are naturally occurring, readily available, structurally characterized and elicit positive biological effects at behaviorally achievable non-toxic concentrations. Our lab has been investigating the use of a cranberry proanthocyanidin rich extract (C-PAC) as a preventative agent targeting esophageal adenocarcinoma (EAC) and premalignant Barrett's (BE) cells. Rates of EAC and the only known precursor lesion, Barrett's esophagus (BE), have markedly increased over the last three decades throughout the Westernized world. In the United States, 16,980 new incident cases and 15,590 deaths have been estimated in 2015 by the American Cancer Society. These statistics reflect the poor 5-year survival rates (20%) and support the need for improved preventive interventions.

We recently reported for the first time that C-PAC induces autophagic cell death in apoptotic resistant EAC cells and necrosis in EAC cells that are also autophagy resistant. Thus, the current investigation sought to investigate the role of reactive oxygen species generation and PI3K/AKT/mTOR signaling in the context of C-PAC induced cell death induction utilizing a panel of human BE and EAC cell lines. In brief, we sought to characterize C-PAC's effect on reactive oxygen species (ROS), to identify the species of ROS induced by C-PAC and to monitor gene expression changes following C-PAC treatment of BE cells and EAC cells. CellROX® Green reagent was used initially to monitor ROS levels, but we are now proceeding with Amplex red and more sensitive HPLC based assays to evaluate specific ROS species. We examined ROS levels of BE and EAC cell lines following exposure to C-PAC at 25 and 100 µg/ml alone and in combination with the pro-oxidant tert-Butyl hydroperoxide (TBHP) or the anti-oxidant N-acetyl cysteine (NAC). High dose C-PAC treatment significantly increased ROS levels at 3 hours (3.0 to 8.1-fold increase) and 6 hours (3.4 to 10.8-fold increase) in all EAC cells evaluated. In contrast, C-PAC significantly reduced ROS levels in CP-C BE cells (4-8-fold at 6 H); yet, C-PAC reduced viability of all cells 24-48 H post treatment. TBHP strongly induced ROS only in JHAD1 cells (21.2-fold) resulting in rapid death. NAC had the strongest mitigating effects in OE19 cells; the only cells which readily formed xenografts *in vivo*. These data suggest that C-PAC induces cancer cell death via ROS induction in EAC cells and ROS inhibition in premalignant cells. Collection of cell lysates from C-PAC treatment of EAC cells showed inactivation of PI3K/AKT/mTor signaling networks as evidenced by total loss or significant reduction of P-p70S6k, P-Akt^{Thr308} and P-Akt^{Ser473} expression in all EAC cell lines 24 and 48 hours post C-PAC treatment. mTOR levels also decreased following C-PAC treatment. The results support C-PAC inhibition of mTORC1 and mTORC2 signaling. C-PAC induced modulation of gene expression focused on pathways of cell death, autophagy, oxidative stress and antioxidant defense will also be presented from BE and EAC cells treated with C-PAC. Collectively, these experiments will provide additional mechanistic insight regarding C-PAC induced cancer cell death through induction of ROS species and modulation of multiple signaling pathways linked to autophagy induction.

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Intake of Whole Raspberries and the Raspberry Phytochemicals, Ellagic Acid and Raspberry Ketone Reduce Adiposity, Improve Glucose Control and Changes Hepatic Gene Expression in High-fat Fed Mice

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A significant body of research demonstrates that intake of berries and berry phytochemicals favorably influence metabolism via a number of different biological mechanisms. The current study tested the relative effect of whole raspberry products or raspberry phytochemicals using the C57BL/6J mouse fed a high-fat, high-sucrose diet as a model for the obesigenic Western diet (HF). Diets were formulated to include a typical dietary level of whole raspberry food products, or for the case of ellagic acid and raspberry ketone, levels that could be reasonably achieved with a few servings of food or typical dietary supplement use. Animals were fed experimental diets ad libitum and body weight and food intake was recorded weekly. In weeks six, a glucose tolerance test was conducted. After ten weeks, animals were killed, serum collected, and liver tissue saved for RNA isolation and gene expression analysis. The results of the glucose tolerance tests indicated that Area Under the Curve (AUC) was similar for several groups of high-fat fed mice fed diets supplemented with whole raspberry powder and raspberry phytochemicals as compared to healthy mice fed a low-fat (LF) diet. Similarly, weight gain for these raspberry groups was decreased and in all groups, intermediate in value to the modest weight gain of the LF-fed mice and the profound weight gain of HF-fed mice. Finally, a custom gene array was used to evaluate the expression of 87 different genes, related to lipid metabolism and transport, carbohydrate and lipid metabolism, inflammation, and gene transcription factors. A significant number of genes were found to be up- and down-regulated ($p < 0.05$) in HF-fed mice compared to LF-fed mice. Interestingly, the consumption of whole raspberry products, ellagic acid, and raspberry ketone altered the relative mRNA levels for these genes so that the pattern of expression more closely resembled that of the mice fed a LF diet. We conclude that although many biological mechanisms may be responsible, clearly, hepatic gene expression is being altered in a favorable manner with the consumption of raspberries and raspberry phytochemicals.

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A pilot study to test the effect of dietary factors on strawberry anthocyanin bioavailability and metabolic indices

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Epidemiological and clinical studies have shown health benefits of consuming strawberries. Strawberries are high in polyphenolic compounds, primarily anthocyanins (i.e., pelargonidin), and others including flavan-3-ols (i.e., catechin), flavonols (quercetin and kaempferol), and ellagitannins. Dietary anthocyanins have received increasing attention for their health benefits. The bioavailability of anthocyanins is low in general; however, various dietary factors may impact bioavailability further. Milk is suggested to impact (poly)phenol bioavailability because of their affinity to milk proteins. The effect of milk on anthocyanin bioavailability remains uncertain. Likewise, mixed nutrient meals may influence bioavailability of anthocyanins. The purpose of the present study was to determine if milk impacts the bioavailability and metabolic profile of strawberry anthocyanins consumed with and without a meal. Using a cross-over study design, nine healthy participants consumed a formulated strawberry beverage prepared in milk or water along with a standard high carbohydrate, moderate fat meal. To determine the impact of the meal, the same beverage formulas were given to a subset of subjects (n=4) on two other occasions without the meal. Blood samples were collected at 0, 0.25, 0.5, 1, 1.5, 2 hours, then hourly until 6 hours to assess anthocyanin percent bioavailability and pharmacokinetic parameters in response to experimental conditions. Postprandial changes in plasma glucose, insulin and lipids were also assessed. Preliminary data indicate differential bioavailability between strawberry anthocyanins, but no significant difference in total anthocyanin bioavailability between milk and water formulas when consumed with a meal. Similarly, no significant differences were observed in the 6-hour postprandial metabolic responses between milk and water conditions in the meal setting: glucose (597.6 mg/dL/h ± 20.5 vs 602.5 mg/dL/h ± 30.3); insulin (221.9 uIU/mL/h ± 32.5 vs 235.7 uIU/mL/h ± 40.4), and triglyceride (540.1 mg/dL/h ± 93.7 vs 553.6 mg/dL/h ± 82.6). Sample analyses are in progress and the full set of data will be presented.
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Identification of anthocyanin metabolites in human plasma after consuming a wild blueberry drink

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Diets abundant in phenolic compounds have been linked with a decreased risk of cardiovascular disease, obesity and diabetes. Wild blueberries are one of the richest sources of dietary phenolic compounds, particularly anthocyanin compounds. They also contain hydroxycinnamic acids, namely chlorogenic acids. Understanding their metabolic fate may provide insight to their health benefits and protection against chronic disease development. The purpose of this study was to evaluate the metabolite profile of wild blueberry anthocyanin and chlorogenic acid in human plasma. Healthy male and female volunteers (age 20-35 years) were enrolled for this study. Plasma samples were collected before (t=0h) and 2h after consumption of wild blueberry drink (25g freeze dried wild blueberry powder) with a standard polyphenol free breakfast. Metabolites in plasma were extracted using solid-phase extraction technique before analysis. The anthocyanins in wild blueberry drink and their metabolites in human plasma were identified using HPLC-ESI-Q-TOF and confirmed by MRM transition on HPLC-ESI-QQQ. A total of 29 anthocyanins were identified in the wild blueberry drink. These included glycosides of cyanidin, delphinidin, malvidin, petunidin and peonidin, which were detected in positive mode. Glucose was the most abundant glycoside attachment followed by galactose, arabinose, acetylated glucose, acetylated galactose and xylose. Chlorogenic acids (CGA) were also found in the wild blueberry drink in negative mode and confirmed with standards. 3-CGA was the most abundant among the three types of CGA. All the intact forms of anthocyanins and 3-CGA were found in human plasma in the 2 h sample. Phase I and phase II metabolites, including malvidin-glucuronide, peonidin-glucuronide, pelagonidin-3-glucoside, delphinidinglucuronide, cyanidin-glucuronide, phloroglucinaldegyde, 3,4-dihydroxybenzoic acid were also identified in 2h plasma samples. These results suggest that anthocyanins and chlorogenic acid from wild blueberry drink can be absorbed in the human body in their intact form.

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Potential use of berry tannins combined with chitosan for developing new composite biomaterials for medical applications

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Chitosan has been applied to promote extracellular matrix (ECM) formation in tissue regenerative therapy. The superior tissue compatibility of chitosan may primarily be attributed to its structural similarity to glycosaminoglycan in ECM. Chitosan has been reported to be biocompatible, bio-absorbable and particularly, is considered a good wound-healing accelerator. Dermis and scaffolds made from chitosan exhibit weak antigenicity, biodegradability, and superior biocompatibility (hemostatic and cell-binding properties) by comparison to the synthetic polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and polyethylene terephthalate (PET). As a scaffold, chitosan-based materials in the form of a sponge have been considered the most popular 3D-scaffolds for dermal regeneration. Of the many scaffold materials being investigated, berry tannins have been shown to have many advantageous features. Highly porous berry tannins lattice sponges have been used to support in vitro growth of many types of tissues.

We isolated chitosan from native shrimp waste streams and isolate berry tannins from cranberry. Hybrid 3D-scaffold biomaterials were successfully obtained by mixing chitosan with berry tannins at different molar ratios. Chitosan-Tannins hybrid composites were formulated as 3D sponge-like scaffolds, applying previously developed methodologies involving solvent casting and freeze-drying. Chitosan-Tannins hybrid 3D-scaffolds were characterized according to its thermal behavior (DSC) and morphology (SEM).

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Chemical constituents and *in vitro* modulation of NF-κB activity of European elderberries

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Black elderberry (*Sambucus nigra* L.) has been used as a medicinal plant from ancient times to defend cold, flu and many other diseases. The berries are rich in polyphenols, and in particular anthocyanins. The most abundant anthocyanins in European black elderberry is cyanidin-3-sambubioside-5-glucoside and cyanidin-3-glucoside. Other polyphenols are chlorogenic acid and quercetin glycosides.

In this study, we have examined six different European varieties of black elderberry: Samidan, Samyl, Samidal, Sampo, Sambu and Samnor. They were grown at the research station Njøs at Leikanger along the Sognefjord in the western part of Norway, and harvested fall 2013 and fall 2014. The summer 2013 was cold with a lot of rain, while summer 2014 was much warmer and sunnier. The berries were analyzed We for ascorbic acid, total phenolics (TP), total monomeric anthocyanins (TMA), sugars, acids, pH, Brix, individual anthocyanins and other phenolic compounds. In addition, immune response of the berries, different fractions of the berries and individual chemical compounds were determined. This was performed by use of humane monocytic cell-lines U937 3xκB-LUC. The modulation of basal and LPS-induced NF-κB-activity was measured. NF-κB is a gene regulatory protein, which control many of the immunologic responses in a cell. Measuring change in the NF-κB activity in cells after stimulation of a compound can give valuable information about the immunological effect of the compound or of a product.

The results showed significant differences in concentration of anthocyanins between varieties and between years for some of the varieties of black elderberry. Similar results were obtained for immunological response, determined as modulated LPS-induced NF-κB activity.

Ascorbic Acid-Catalyzed Degradation of Cyanidin-3-Glucoside: Proposed Mechanism and Identification of a Novel Hydroxylated Product

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Many brightly colored fruits and vegetables owe their pigmentation and beneficial health effects to anthocyanins. Unfortunately, anthocyanins are readily degraded over juice processing and storage, which adversely affects color stability and potential health benefits. This project focused on the effect of ascorbic acid as a catalyst in anthocyanin degradation. The first step of the project involved searching for novel pigmented compounds in a simple model system composed of the most common anthocyanin cyanidin-3-*O*-β-glucoside and ascorbic acid. Over 72 hours at ambient temperature, 67% of cyanidin-3-glucoside was lost in this system during which time an unknown pigmented compound was formed. A follow-up study confirmed the emergence of the unknown compound in a more complex blackberry extract supplemented with ascorbic acid. HPLC with PDA monitoring at 510 nm was used to detect the novel compound and LC-ESI-MS^3 allowed a proposed structure to be built based on the fragmentation patterns. The unknown structure formed via oxidation of cyanidin 3-glucoside by ascorbic acid was identified as 6-hydroxy-cyanidin-3-glucoside. We propose that the pigmented compound is formed from hydroxyl radicals via the Haber-Weiss reaction.

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Inhibitory effects of common edible berry extracts on the formation of advanced glycation endproducts

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Glycation is a spontaneous process between reducing sugars and proteins that leads to the formation of Advanced Glycation Endproducts (AGEs). The formation and *in vivo* accumulation of AGEs have been linked to several chronic human diseases such as diabetes, inflammation, and neurodegenerative diseases. Current data suggests that phenolic-rich fruit, such as berries, show great promise as natural anti-AGE agents. Moreover, recent studies have shown that wild berries exert anti-glycation activity which correlates with their antioxidant activities and total phenolic content. However, there is no similar data on commonly cultivated edible berries including black raspberry (*Rubus occidentalis*), blackberry (*Rubus sp.*), blueberry (*Vaccinium angustifolium*), cranberry (*Vaccinium macrocarpon*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*). Therefore, these six berry powders were dissolved and extracted using an XAD-16 column to yield anthocyanin-free (ACF) and anthocyanin-rich (ACR) fractions which were subjected to different assays: DPPH (antioxidant), total phenolic content, total anthocyanin content, methylglyoxal scavenging assay and anti-AGE assays. The fractions were evaluated for anti-AGE effects by an intrinsic fluorescent assay using bovine serum albumin (as the model protein) and D-fructose (as the glycating agent) and compared to Aminoguanidine, a synthetic anti-AGE agent (56 % inhibitory effect at 100 μg/mL). At equivalent concentrations of 100 μg/mL, the ACR extracts of black raspberry, blackberry, blueberry, cranberry, red raspberry and strawberry were 150.6, 36.8, 60.69, 59.66, 41.3 and 37.4 %, respectively. The ACR fractions had stronger inhibitory effects on AGE formation compared to their respective ACF fractions. This study suggests that anthocyanins are the major contributors to the anti-AGE activities of these common edible berries.

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Effects of Berry Processing into Whole Berry Puree's or Smoothies on Multi-Radical Antioxidant Capacity (ORAC-MR_5)

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Berries have been shown to be an excellent source of antioxidants in the diet. This informatio comes primarily from the use of the peroxyl radical in the Oxygen Radical Absorption Capacity (ORAC) assay. Recently, methods of assay have been developed using other biologically relevan radical/oxidant sources for assessing *in vitro* antioxidant capacity. Berries are often processed and marketed in various forms for bakery, jam, or jelly use or pureed for juice/pulp manufacture. Depending upon the processing method(s), antioxidant capacity can be altered, but no data is available using multiple radical sources.

The objective of this study was to determine the relative antioxidant capacity of wild blueberries, mango and cherries using 5 different biologically relevant free radicals/oxidants and the effects of processing into a whole fruit puree or blueberry smoothie. $ORAC_{MR5}$ methods described by Nemezer and Ou(*1*) were used for antioxidant capacity assay using peroxyl (ORAC), hydroxyl (HORAC), superoxide anion (SORAC), peroxynitrite (NORAC), and singlet oxygen (SOAC) radicals/oxidants. Smoothies [Blueberry/pomegranate (BPS) and Mango/pineapple MPIS)] were obtained from a local McDonalds restaurant. BPS contained blueberries, raspberries and pomegranate juice (proportions not available) in low-fat yogurt and MPS contained mango and pineapple (proportions not available) in low fat yogurt. Whole berry/fruit purees [Mango Passion Fruition (MPF); Wild Blueberry Fruition (WBF); Cherry Fruition (CF) were obtained from Wyman's of Maine (Milbridge, ME).

Total $ORAC_{MR5}$ expressed as µmoles TE per serving and the per cent contribution of ORAC, HORAC, SORAC, NORAC and SOAC to the total $ORAC_{MR5}$ is presented in the table below.

	ORAC, %	HORAC, %	SORAC, %	NORAC,%	SOAC, %	$ORAC_{MR5}$ *
Smoothie						
BPS	24.8	4.5	1.0	0.0	69.7	8931
MPIS	12.3	2.9	7.4	0.0	77.4	9284
Puree						
MPF	1.5	3.6	0.1	0	95.0	19319
CF	6.3	7.3	0.3	1.1	84.9	36931
WBF	14.5	14.3	1.0	2.7	67.4	29275
Wild BB	42.0	22	4	4	28	

* Total $ORAC_{MR5}$ µmoles TE for a serving size of 265 mL for smoothies and 87 g for whole fruit purees

Data from fresh wild blueberries (Wild BB) is presented as a comparison. The remarkable observation is the relatively high singlet oxygen capacity in processed samples and >17% lower amounts for ORAC and HORAC in the processed samples relative to the Wild BB sample. Comparing the unprocessed Wild BB to WBF, on a µmole basis, the increase in SOAC can be accounted for by the decrease in ORAC and HORAC. Blackberry is the only berry studied to this point that has a predominance of singlet oxygen antioxidant capacity. Additional studies are needed to determine the conditions responsible for the changes in antioxidant capacity within $ORAC_{MR5}$

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Development of a confection with blueberry extract directed at improving cognitive health of children post-chemotherapy

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With recent advances in treatment the survival rate of childhood cancer exceeds 80%,[1] such that 1 in 640 young adults in the United States is estimated to be a pediatric cancer survivor. A common and potentially debilitating late effect of childhood cancer treatment is neurocognitive impairment, particularly executive dysfunction, which can limit educational attainment and future employment.[2] Given the potential of pervasive impact of neurocognitive impairment on daily life, interventions directed at reducing neurocognitive dysfunction among long-term survivors of childhood cancer are needed.

Our objective is to examine the safety and effectiveness of an intervention using food based matrix to deliver blueberry anthocyanins combined with a supplement of n-3 fatty acids on longitudinal changes in cognitive performance among survivors of childhood acute lymphoblastic leukemia (ALL) post chemotherapy. Based on the multiple etiologies, varying manifestations and extent of cognitive decline documented in ALL survivors, we hypothesize that interventions using a food based matrix of the extract containing anthocyanins [3-6] in combination with n-3 fatty acids [7-11] supplement (COGNUTRIN®) may work synergistically to facilitate reductions in oxidative stress loads and inflammatory cytokines, with significant improvement in cognitive health resulting in improvements in quality of life of childhood acute lymphoblastic leukemia survivors. In addition, a food based matrix may improve acceptability of COGNUTRIN® for use in children.

Confection based matrices have been applied in human clinical studies to improve the acceptability and delivery of the bioactive compounds from black raspberry in our previous study[12], thus a confection with blueberry extract enriched in anthocyanins was developed to be used in this trial to ensure trial feasibility, tolerance to daily use, adherence to a multiple dose administration schedule in this pediatric patient population as young as 8 years of age. A delivery dosage of 0.5 g extract per piece of confection was achieved with consideration of acceptability, storage stability, and anthocyanin retention. Further works will focus on the confection development of the combination of anthocyanins and n-3 fatty acids.

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Development of a confection matrix for the delivery of galacto-oligosaccharides and black raspberry phytochemicals

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Extensive research has shown that the gut microbiota plays a key role in human health. Factors such as diet, stress, illness, and antibiotics can cause a microbial imbalance, leading to a number of chronic conditions. Prebiotics are non-digestible ingredients that selectively stimulate growth of beneficial gut bacteria, and thus are of great interest for intervention studies. Compounds with prebiotic potential include the non-digestible carbohydrates, galacto-oligosaccharides (GOS), and phenolic compounds present in certain fruits. Black raspberries are rich in phenolic compounds, including anthocyanins, quercetin, and ellagic acid which have potential to alter gut microbial composition, in addition to their antioxidant properties that may play a preventative role in cancers and other chronic diseases. Our objective was to develop a matrix to deliver GOS in conjunction with whole, freeze-dried black raspberries, which will allow future studies to investigate a potential synergistic effect of the compounds to both alter the population of gut microbiota and improve the bioavailability of phenolic compounds. Research has shown that non-digestible carbohydrates may act synergistically with phenolic compounds to improve their bioavailability by aiding in transport to the large intestine, and by altering gut physiological function in order to more effectively transform phenolic compounds into metabolites of health significance. We have developed an amorphous glassy confection with acceptable physiochemical properties consisting of 25% GOS, the GRAS approved level for sugars and sweets, which entraps 20% black raspberry powder. A glassy confection is suitable for controlled and sustained release rates of bioactive compounds. Confections, such as the one developed, with high sensory acceptability, is a convenient delivery system that can be easily incorporated into a daily diet resulting in increased compliance in future human clinical trials.

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Multi-Radical Antioxidant Capacity (ORACMR) of Selected Berries

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The capability of using multiple free radical sources is an advantage of the Oxygen Radical Absorbing Capacity (ORAC) method. In addition to the original ORAC assay using the peroxyl radical developed by Ou and Huang, Prior et al (1, 2), a new panel of ORAC assays have been developed by chemists at International Chemistry Testing (Milford, MA), using the reactive oxygen species, superoxide anion, hydroxyl radical, and singlet oxygen, and the reactive nitrogen species, peroxynitrite. This panel of assays has led to the term, Oxygen Radical Absorbance Capacity using Multiple Radicals (ORACMR5) which is a sum of the antioxidant capacity against 5 of the the predominant reactive species found in the human body: ROS (peroxyl radical, hydroxyl radical, superoxide anion, singlet oxygen), and reactive nitrogen species (peroxynitrite).

In the past few years there have been several manuscripts published using the ORAC database demonstrating that increased dietary intake of bioactive/antioxidants as determined using ORAC(Peroxyl) or Total Phenolics by the Folin assay was associated with numerous health benefits (3). It is clear that dietary bioactives/antioxidants have health effects in vivo. Although antioxidant capacity assays provide measures of the relative amounts of 'bioactives' in botanicals, the bioactivity in vivo may not be via strictly antioxidant mechanisms. Assessing the antioxidant capacity of a given food by means of an in vitro assay does not imply that the total activity of its antioxidant components will become bioavailable to the organism. It is important to realize also that most of the antioxidant phytochemicals in berries and other botanicals can be metabolized by gut microbiota and/or metabolized and conjugated during the absorption process. However, using the global index of ORACMR represents a practical form to assess the potential impact that a given food may on in vivo antioxidant status of the organism either by direct or through indirect actions. Lycopene, the red pigment in tomatoes, and other carotenoids are efficient in quenching singlet oxygen. However, utilizing ORACMR, other compounds and foods, including berries are being identified that have significant capacity to efficiently quench singlet oxygen. Because superoxide anion seems to be pivotal in the formation of other free radicals, ORACMR provides for the first time the opportunity to identify berries and other foods that have the potential for efficient quenching of superoxide anion and thereby decreasing the oxidative load from other free radicals.

The objective of this study was to determine the relative antioxidant capacity of selected freeze dried berries using 5 different biologically relevant free radicals/oxidants. Data were obtained from 11 different berry sources. Whole berries were freeze dried before analysis for antioxidant capacity, and included Blackberry, Wild Blueberry, cultivated organic Blueberry, Elderberry, Bilberry, Boysenberry, Strawberries, Sweet cherry, Tart cherry, Black Currants and Raspberry. Total ORACMR5 ranged from 475 to 3003 µmoles Trolox Equivalents per g dry weight for the berries analyzed (See Table). Singlet O2 antioxidant capacity contributed to more than 60% of the ORACMR5 in blackberries, sweet and tart cherries and no singlet O2 AOC found in strawberries, black currants and raspberries. Although it has previously thought that more antioxidant capacity is better, it may be that the right balance of antioxidants may be critical. A score has been developed reflecting the "balance" of antioxidants against the different radicals in each of the berries. Using this information it is possible to select a combination of berries that will complement each other and provide a reasonable "balance" of antioxidants in the diet.

Cont.

Table: Antioxidant capacity against multiple radicals and fruits and berries*****

Berry/Fruit	Peroxyl (ORAC)	Hydroxyl HORAC	Peroxy-nitrite	Superoxide Anion (SORAC)	Singlet O2 (SOAC)	TOTAL $ORAC_{MR5}$
Blackberry	423	99	31	478	1971	3003
Acai, E. fruit pulp	986	1357	17	169	71	2600
Blueberry, Wild, 2013	880	823	71	189	386	2349
Blueberry, organic, FD	618	105	47	562	477	1809
Elderberry, whole, FD	953	143	60	208	329	1694
Bilberry, whole	767	112	58	411	308	1655
Boysenberry, FD	216	76	35	391	495	1212
Blueberry, Wild, 2014	509	269	44	45	339	1206
Strawberry	356	101	26	239	0	722
Cherry, sweet	202	125	6	26	504	864
Cherry, tart	149	16	10	77	520	772
Black Currants	389	65	31	258	0	742
Raspberry	161	60	22	232	0	475

* Data expressed as µmoles Trolox Equivalents per g dry weight.

Examples will be presented as to how consumption of different combinations of berries can be used to provide a “balanced” group of antioxidants in the diet. Raspberries, Black Currants, Strawberries and Boysenberries had 30-50% of their total $ORACR_{M5}$ as superoxide anion antioxidant capacity (SORAC) and thus may have greater potential to suppress the oxidative load from other radicals. More research is needed on how these different radicals alter the antioxidant status of the body.

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Bioprospecting evaluation of three genotypes of BlackBerry (*Rubus adenotrichos*) cultivated in the Trinidad of Copey, Dota, Costa Rica

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Abstract

Blackberries (Rubus spp.) are fruits rich in nutraceuticals components (anthocyanins, proanthocyanidins, phenolic acids, tannins, carotenoids and others), recognized for its health benefits. The objective of this research consisted of an assessment in the content of vitamin C, phenolic compounds and antibiotic and antioxidant activity present in the crude extract of three genotypes of blackberry (*Rubus adenotrichos*) collected in the Trinidad of Copey, Dota. These genotypes were identified as red thorns, without thorns and sweet.

The fruits were analyzed in three stages of maturation (green fruit, red fruit and black fruit) and the leaves in two stages of maturation (young leaves and old leaves). Column chromatography packed with C-18 and Sephadex LH-20 resins were used to obtain standards of polyphenols and proanthocyanidins respectively. Analysis using HPLC, LC-ESI-MS and NMR-H revealed that the principal anthocyanin present in our *Rubus adenotrichus* samples was the Cyanidin-3-glucoside. Also, it was observed that the red thorns genotype presented the best results with a concentration of polyphenols of 183.0 ± 0.5 mg eq of Gallic Acid/g of dry sample, antioxidant capacity of 3322 ± 10 μmol of Trolox eq/g of dry sample , a value of 15.4 ± 0.3 mg eq Cyanidin-3-glucoside/g of dry sample of anthocyanins, and a value of 9.26 ± 0.03 mg eq 4´-methyl-galocatequina/g of dry sample for the content of proanthocyanidins, for vitamin C the content was 363 ± 2 mg of eq of Ascorbic Acid/g of dry sample. Finally, for the antibiotic activity presented a percentage of inhibition of 83.3% ± 0.4 of the bacterium S. areus. As a conclusions, the red thorns genotype of Costa Rican blackberry has all the features for the development of nutraceuticals, cosmetics and medicines, thanks to these characteristics it is classified as a superfruit. It should be disclosed properly and to publicize these findings to the Costa Rican population, both producers as consumers so that promotes the cultivation and consumption of this red thorns genotype in Costa Rica could thus reduce the high incidence of patients with cancer and other diseases and achieve high levels of longevity in the country.

Keywords

Nutraceutic, Folin-Ciocalteu, anthocyanin, proanthocyanidins, trolox, blackberry.

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Anti-angiogenic activity of red raspberry cyanidin-3-sophoroside

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Abstract

Type 2 diabetes is an angiogenic disease whereby insufficient and excessive angiogenesis can exist in the same individual. Red raspberry is a rich source of dietary bioactives including anthocyanins, anthocyanidins, and ketone bodies. We tested the hypothesis that cyanidin-3- sophoroside, the major anthocyanin in red raspberry may inhibit angiogenesis. In vitro inhibition of angiogenesis was studied by (i) endothelial tube formation on Matrigel, (ii) endothelial cell migration and invasion and (iii) expression of angiogenic factors in the presence or absence a various concentrations of cyanidin-3 sophoroside. The results were statistically analyzed by analysis of variance. Cyanidin-3-sophoroside at concentration between 1 and 200 µM demonstrated significant dose-dependent anti-angiogenic activity in human umbilical vascular endothelial cell (HUVEC) based on (i) inhibition of tube formation in Matrigel, (ii) inhibition of cell migration and invasion and (iii) inhibition of the expression of pro-angiogenic growth factors. These in vitro results provide a mechanistic evidence to substantiate the in vivo anti-angiogenic property of edible berries reported in the literature.

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Nutraceutic study for Costa Rican blackberry (*Rubus sp)*: total phenolic compounds and antioxidant activity as indicators of plant quality produced in Costa Rican highlands

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In the present work, total phenolics and antioxidant activity of different structures of the *Rubus sp.* such as stem, leaves and berries at three stages of ripeness (green, red and purple) were evaluated. In order to accomplish the purpose of the study, crude extracts of the different substrates were tested for total phenolics using Folin-Ciocalteu method and gallic acid and a Crude Polyphenol Extract of Blackberry (CPEB) as references to report the data. Antioxidant activity using DPPH assay was performed for the same crude extracts. Finally, isolation of anthocyanins was carried out for the red and purple fruit produced in San Martin, Leon Cortez.

The assays showed total phenolics and anthocyanins decreased as the maturation state progressed, revealing higher concentrations of these compounds in younger fruit structures. Blackberry leaves have the highest level of total phenolics among plant tissues, followed by the green fruit, stem, red and purple fruit.

Comparing the results of total phenolics obtained using gallic acid and CPEB, it shows that the first one underestimated the amount of total phenolics found in Costa Rican blackberry plants, leaving aside, at least 90 mg/g on a dry weight basis.

A significant relationship between total phenolic compounds and antioxidant activity was found ($r=0.965$; $P<0.01$) showing that the high antioxidant activity in different parts of the plant is closely related to the plant total phenolic concentration.

Anthocyanins did not appear to be the predominant contributor to antioxidant capacity in Costa Rican blackberry fruit. This conclusion was based on the finding that red fruit had higher antioxidant capacity than purple fruit, while an inverse trend was observed for anthocyanins.

Keywords

Nutraceutic, Folin-Ciocalteu, DPPH, anthocyanin, blackberry.

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Inhibition of Formation of Advanced Glycation End-products by an Oligosaccharide-Enriched Fraction Purified from Cranberry (*Vaccinium macrocarpon*)

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Advanced glycation end-products (AGEs) are a polymorphic group of compounds implicated in several chronic complications including type-2 diabetes and neurodegenerative diseases. Herein, an oligosaccharide-enriched fraction, purified from North American cranberries (*Vaccinium macrocarpon*), was evaluated for its anti-AGE effects using human serum albumin and D-fructose as the model protein and glycating agent, respectively. The cranberry oligosaccharide-enriched fraction reduced AGE formation in a concentration dependent manner (44, 47, 61 and 65 % at 10, 50, 100, 200 µg/mL, respectively). Interestingly, 65 % inhibition was achieved with the cranberry oligosaccharides at 200 µg/ml while the positive control, aminoguanidine (a synthetic anti-AGE agent) showed 78 % at a much higher concentration of 500 µg/ml. Further studies to evaluate the mechanisms of anti-AGE effects of cranberry oligosaccharides are warranted and are currently being pursued by our group.

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Notes:

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