



BERRY HEALTH BENEFITS SYMPOSIUM

The only conference focusing solely on berries & human health.

May 7-9, 2019 | Portland, OR - USA

Symposium Pre-Proceedings



The Berry Sessions

Tuesday, May 7th, 2019 - Queen Marie Ballroom



9:00am | Welcome

9:15-10:00am | *Berryology 104* - Dr. Navindra Seeram & Dr. Britt Burton-Freeman - Sponsored by



10:00-10:45am | *From Sound Science to Clear Communication of Nutrition Research* - Leslie Wada, PhD, RD, Dr. Christian Krueger, and Dr. Leslie Redmond - Sponsored by



10:45-11:00am | BREAK

11:00-11:45am | *International Marketing of the Berry Health Message* - Chris Christian, Dr. Carolyn Lister, Christina Khoo, PhD, & Alicia Adler - Sponsored by



11:45-12:30pm | *Berry Nutrient Density Health Research Project* - Dr. Adam Drewnowski - Sponsored by



12:30-1:45 PM | LUNCH - Colonel Lindbergh Ballroom - Sponsored by



Scientific Presentations

Tuesday, May 7th, 2019 - Queen Marie Ballroom

1:50pm | Scientific Presentations Welcome - Chris Christian, National Berry Crops Initiative (NBCI) President

Berries & the Brain (*Pages 18-27*)

2:00-2:15pm | *Current Research Review*. Chair overview by Dr. Barbara Shukitt-Hale

2:15-2:40pm | *The Chronic Effects of a Wild Blueberry Intervention on Cognition and Urinary Metabolites in 7-10 Year Old Children* - Dr. Claire Williams

2:40-3:05pm | *Impact of Raspberries on Vascular Architecture and Cognitive Function in a Transgenic Model of Alzheimer's Disease* - Dr. Chris Gill

3:05-3:30pm | *Impact of Berry Polyphenols on the Gut-Brain Axis* - Dr. David Vauzour

3:30-3:55pm | *The Effects of Berry Polyphenols on Cognitive Function in Adults in the Context of Other Plant Based Ingredients: An ILSI Europe Systematic Review* - Dr. Louise Dye

4:00-5:30pm | Poster Session - Gevurtz Room - (*Pages 70-89*)

Dinner

6:00-8:00pm | BHBS Dine Out - Pine Street Market - No Host



Pine Street Market - 126 SW 2nd Avenue

Scientific Presentations

Wednesday, May 8th, 2019 - Queen Marie Ballroom

Berries & Cardio-Metabolic Health (*Pages 28-37*)

9:00-9:15am | *Current Research Review*. Chair overview by Dr. Britt Burton-Freeman

9:15-9:40am | *Dietary Raspberry Induces Browning of White Adipose Tissue via AMPK* - Meijun Zhu, PhD

9:40-10:05am | *Blueberry Anthocyanins and Proanthocyanins Improve Insulin Sensitivity in Diet-Induced Obese Mice Through Their Impact on the Gut Microbiota* - Dr. André Marette

10:05-10:30am | *Berry Polyphenols and Endothelial function: Findings From Human Studies and Mechanistic Insights* - Dr. Ana Maria Rodriguez-Mateos

10:30-10:55am | *Blueberries for Attenuating Age-Related Vascular Dysfunction: Evidence and Opportunities* - Dr. Sarah A. Johnson

10:55-11:10am | BREAK - Sponsored by  **Driscoll's**
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Scientific Presentations

Wednesday, May 8th, 2019 - Queen Marie Ballroom

Berry Phytochemicals & Metabolites on Cardiovascular Function (Pages 38-43)

11:10-11:25am | *Current Research Review*. Chair overview by Mary Ann Lila, PhD

11:25- 11:40am | *Effects of Short-Term Consumption of Strawberry Powder on Parameters of Vascular Health in Adolescent Males* - Carl Keen, PhD

11:40-12:05pm | *The Impact of Blueberries on Cardiometabolic Health in Participants with Metabolic Syndrome - Results From a 6-Month Trial* - Aedin Cassidy, PhD

12:05-1:30pm | LUNCH BREAK - Colonel Lindbergh Ballroom
Sponsored by



Berry Phytochemicals & Metabolites on Cardiovascular Function (cont.) (Pages 44-47)

1:30-1:55pm | *Dietary Berries, Insulin Resistance and Diabetes* - Arpita Basu, PhD

1:55-2:20pm | *Characterizing the Berry Metabolome: Insights & Examples from Bilberry, Blueberry and Strawberry Interventions* - Dr. Colin D. Kay

Junior Investigator Oral Presentations (Pages 48-50)

2:30-3:30pm | Inah Gu, Natalie VandenAkker, and Claudia Favari

5:00-6:30pm | Poster Session - Gevurtz Room - (Pages 70-89)

Keynote Dinner - Queen Marie Ballroom

6:30-8:30pm | *Berries – They're Not Just for Breakfast Anymore* - Mary Ann Lila, PhD - Sponsored By



Keynote Address

Wednesday, May 8th, 2019 - Queen Marie Ballroom



Mary Ann Lila, PhD

North Carolina State University

Berries – They’re Not Just for Breakfast Anymore

Talk Abstract | Berries have come a long way since their traditional role as a garnish atop bland breakfast cereals. Thanks in part to social media-sharing and popular press, today’s consumer realizes that berries can have a starring role in just about every course in every meal of the day. In addition, thanks to their resilient and versatile phytoactive properties, berries have now gained a strong foothold in applications well beyond their usual dietary and functional food uses. This presentation will describe the 1) potent skin-protective properties of berry components (especially as a shield against the modern insults of pollution and UV light), 2) the synergistic relationship between berry polyphenols and human physical exertion to ease exercise-induced inflammatory stress, and 3) innovative new ways to deliver concentrated berry phytoactive compounds in shelf-stable snack formats.

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

Scientific Presentations

Thursday, May 9th, 2019 - Queen Marie Ballroom

Gut Health & Gut Microflora (*Pages 52-61*)

9:00-9:15am | *Current Research Review*. Chair overview by Dr. Jess Reed

9:15-9:40am | *Phenyl- γ -valerolactones and Phenylvaleric Acids, the Main Colonic Metabolites of Flavan-3-ols: Their Relevance in Berry Health Benefits* - Daniele Del Rio, PhD

9:40-10:05am | *Impact of Cranberry Juice Consumption on Gut and Vaginal Microbiota in Post-Menopausal Women* - Dr. Franck Carbonero

10:05-10:30am | *Promoting Colon Health by Berries: the Case Studies of Strawberry and Cranberry* - Dr. Hang Xiao

10:30-10:55am | *The Tannin Fractions of Polyphenol Berry Extracts Regulate Glycemia Through a Modulation of the Gut Microbiota and an Improvement of Gut Barriers Function* - Dr. Yves Desjardins

11:00-11:15am | BREAK - Sponsored By



Berry Special Topics, Food Technology & Chemistry (*Pages 62-69*)

11:15-11:30am | *Current Research Review*. Chair overview by Dr. Navindra Seeram

11:30-11:55am | *Anti-proliferative Activity of Berry Volatiles* - Luke Howard, PhD

11:55-12:20pm | *Sweet Health Prospects for Cranberry Sugars* - David Rowley, PhD

12:20-12:45pm | *Are We Ready for Precision Cancer Prevention Using Black Raspberries?* - Li Shu Wang, PhD

12:45pm | BOXED LUNCH BREAK

1:30-8:30pm | Oregon Berry Discovery Tour

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Tuesday, May 7th - The Berry Sessions

Berryology 104

Understanding the Terminologies Used in Speaking about Berry Health



Navindra Seeram, PhD
University of Rhode
Island

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

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

His research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants and their derived natural products for preventive and therapeutic effects against chronic human diseases. Dr. Seeram has co-authored over 165 original peer-reviewed research articles, 10 review-type articles, 17 book chapters, and 6 international patents. He has co-edited 3 books and is the founding editor of the Clinical Pharmacognosy book series published by CRC Press/Taylor and Francis. He serves on the advisory board of the American Botanical Council and on the editorial advisory boards of the Journal of Agricultural and Food Chemistry, the Journal of Berry Research, and the International Journal of Applied Research in Natural Products.



Dr. Britt Burton-Freeman

Illinois Institute of
Technology

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

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

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

From Sound Science to Clear Communication of Nutrition Research

How do we ensure that the berry health message communicated to consumers is clear and accurate? By starting with sound science and ensuring that down the line it is delivered with clear communication.



Leslie Wada, PhD, RD
Research Administrator
for the US Highbush
Blueberry Council

Leslie Wada, PhD, RD, has worked with the US Highbush Blueberry Council (USHBC) for the past twelve years as the Research Administrator for the Health Research Committee.

She received her Bachelor of Science degree in Nutrition and Dietetics from the University of California at Davis. After graduation from Davis she completed a dietetics internship at UC San Francisco Medical Center to become a registered dietitian. She then went on to receive a PhD in Human Nutrition from UC Berkeley and remained at Berkeley to conduct research and teach undergraduate courses in nutrition and dietetics.

She left UC Berkeley to work in industry and was a founding partner in a startup company that developed nutraceutical ingredients. She then started an independent consulting business and worked as a consultant for companies in the food and ingredient industry which eventually led her to the USHBC.



Dr. Christian Krueger
CEO of Complete
Phytochemical
Solutions, LLC

Dr. Christian 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.

Christian 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. Christian 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.



Dr. Leslie Redmond

Assistant Professor,
University of Alaska
Anchorage

Dr. Leslie Redmond is a registered dietitian with a masters degree in kinesiology and a doctorate degree in nutrition from Johns Hopkins Bloomberg School of Public Health. She is currently an Assistant Professor of Dietetics and Nutrition at the University of Alaska Anchorage, where her research focuses on the promotion of nutrition and physical activity to decrease chronic disease burden in Native North American populations. She also has worked for the California Strawberry Commission where she oversaw the commission's strawberry health human clinical trial research program and served as a liaison to the professional health community, relevant trade associations, key nutrition opinion influencers, and consumers.

Dr. Redmond is dedicated to the responsible communication of scientific literature to the general public and believes that we all play a part in protecting consumers from misinformation and in promoting sound, evidence-based nutrition and health messages.

International Marketing of the Berry Health Message

The differences of marketing the message of berries and health to different parts of the world.



Chris Christian
Vice President of the
California Strawberry
Commission

Chris Christian serves as senior vice president at the California Strawberry Commission where she oversees financial operations and domestic and international marketing programs, including a nationally recognized health research program. Prior to her sixteen years at the Commission, Chris worked in management positions in the food and agriculture industries, including ten years at Fresh Express Inc., where she held key roles in operations, research, and business management. Throughout her career, she has established relationships with produce professionals in the retail and food service industries, world class researchers, and food safety professionals.

Chris holds a master's degree in food science and bachelor's degree in microbiology from the Pennsylvania State University. She is president of the National Berry Crops Initiative, a Board Trustee and Health and Wellness Committee co chair of the Produce for Better Health Foundation and has served in leadership roles with other produce industry trade associations. She is a member of the Institute of Food Technologists (past Chair of Fruit & Vegetable Products Division), and the Academy of Nutrition and Dietetics. In 2018, Chris received an Outstanding Alumna award from the Penn State College of Agricultural Sciences.



Dr. Carolyn Lister
Principal Scientist at the
NZ Institute for Plant &
Food Research

Dr. Carolyn Lister is a Principal Scientist at the NZ Institute for Plant & Food Research and is based in Lincoln. She completed her BSc (Hons) and PhD in plant biochemistry through the University of Canterbury, New Zealand. A fellowship at the University of Glasgow fuelled her interest in the role of plant pigments in human health. Her team works with a range of food and supplement companies and industry groups to quantify nutrients and phytochemicals, such as anthocyanins, and aid in product development.

Dr Lister's expertise extends to developing health claim messages that meet the legal framework of Food Standards Australia/New Zealand (FSANZ), and she understands the current regulatory processes that govern health claim labelling in Australia and New Zealand. She has assisted commercial clients in the collation of health claim dossiers for self-substantiated health claims. More recently her work has extended to the international regulatory environment.



Christina Khoo
Director of Global Health
Science & Nutrition Affairs,
at Ocean Spray Cranberries,
Inc.

Christina Khoo, PhD, is the Director of Global Health Science & Nutrition Affairs, Ocean Spray Cranberries, Inc. and joined the farmer-owned cooperative in 2007.

Christina received her doctorate with emphasis on nutritional biochemistry and mineral metabolism at the Food Science and Human Nutrition Department, University of Florida. She completed a postdoctoral fellowship at Harvard School of Public Health where she conducted human clinical trials in the area of cholesterol and triglyceride metabolism, studying the effects of low or high fat diets on the metabolism and clearance of triglycerides using kinetic modeling.

Her current research interest is focused on cranberry compounds and the antioxidant and whole body health benefits of the cranberries in collaboration with global universities. The research can help to support the role of cranberry in maintaining health as a nutritional approach to reduce antibiotic use. Christina has been instrumental in directing Ocean Spray's effort in supporting research in this area resulting in the generation of a patent application on antibiotic synergy published in July 2017 and one of the largest clinical trial conducted on cranberry and urinary tract health infection, showing a reduction in symptomatic UTI and antibiotic use to treat symptomatic UTI. Christina has directed Ocean Spray's efforts in initiating research in geographies such as China to address health conditions requiring antibiotic use such as H pylori infections. In addition to research, Christina has been active in pulling together stakeholders to continue to evaluate nutritional alternatives.



Alicia Adler
Export Program Manager
for the US Highbush
Blueberry Council

Alicia Adler has served as the Export Program Manager for the United States Highbush Blueberry Council since 2015. Her primary duties include oversight of USHBC's export market promotion programs throughout Asia (Japan, South Korea and Southeast Asia), including grant writing, program and activity development, budget management and government relations.

Prior to USHBC, Ms. Adler served as the Senior Manager of International Marketing and Promotion at Bryant Christie Inc., an international affairs management firm based in Seattle and Sacramento. Bryant Christie Inc. helps companies and organizations open, access and expand international markets with an emphasis on the agricultural, food and beverage sectors. She received her Bachelor of Arts degree in International Relations and Spanish from the University of California at Davis.

Berry Nutrient Density Health Research Project

Nutrient profiling models that incorporate flavonoids put berries on top



Dr. Adam Drewnowski
University of Washington

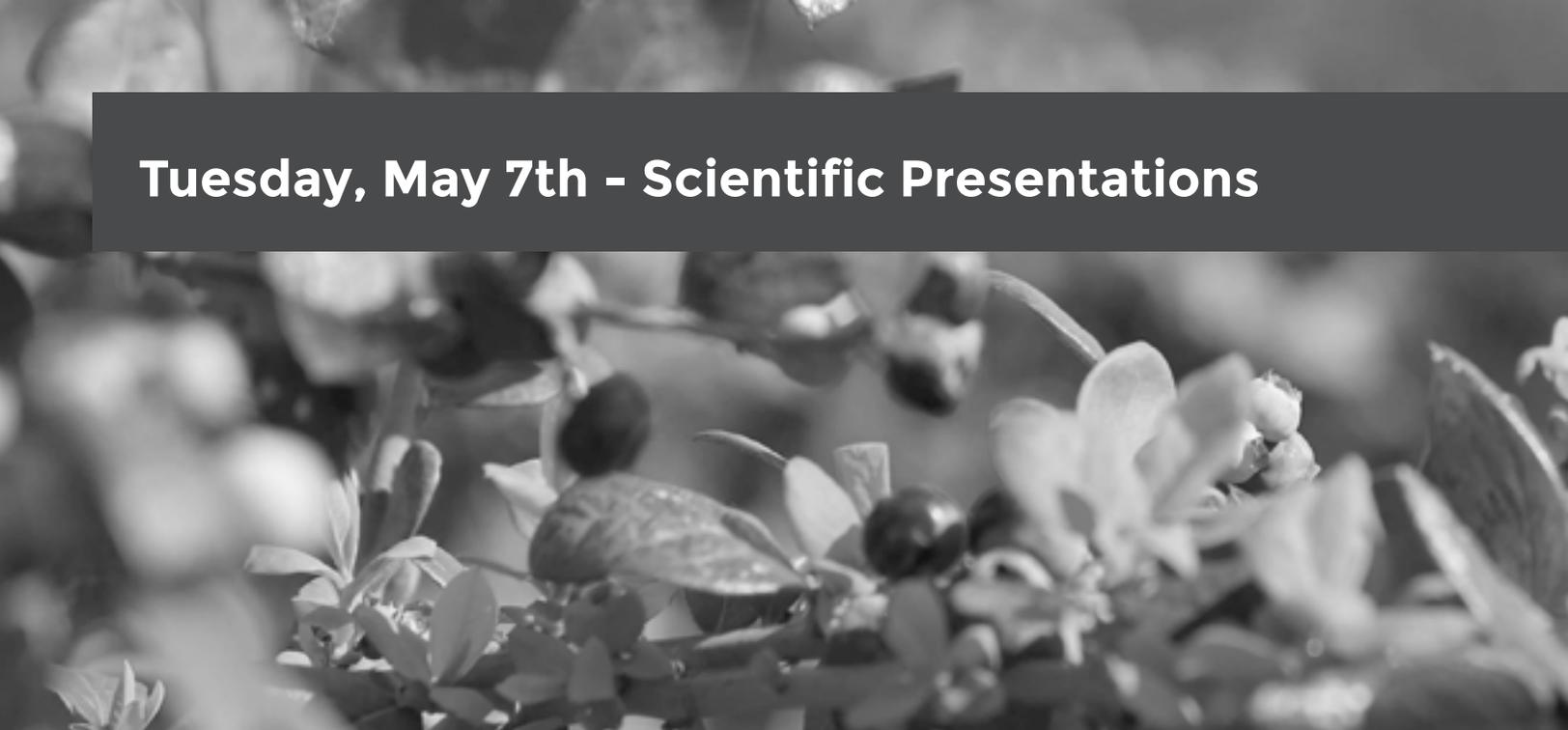
Prof. Dr. Adam Drewnowski is the Director of the Center for Public Health Nutrition at the University of Washington. He obtained his MA degree in biochemistry at Balliol College, Oxford University, and PhD in psychology at The Rockefeller University in New York. He is the author of the Nutrient Rich Foods Index (NRF), a nutrient profiling model, that measures nutrient density of individual foods, meals and composite food patterns. The concept of nutrient density, captured by the NRF index, helps consumers identify foods that are nutrient rich, affordable, and appealing and have a low impact on the environment.

Most recently Dr. Drewnowski has worked on Future 50 foods for healthier people and a healthier planet. Dr. Drewnowski is also the PI of the Seattle Obesity Study (SOS), funded by the National Institutes of Health. The SOS has explored the socioeconomic determinants of health, focusing on access to healthy foods. Using geographic information systems data and geopositioning tracking devices, the SOS has explored where people shop, what they buy, and how their food purchases affect their health and well being. Dr. Drewnowski has authored over 300 research publications. He advises governments, foundations, and the private sector on issues related to diets and health.

Talk Abstract | The concept of nutrient density separates foods that are energy-dense from those that are nutrient-rich. However, current nutrient profiling (NP) methods do not always fully capture all aspects of nutrient density of foods. The two main NP components, fiber and vitamin C do not adequately discriminate among different fruit. The needed expansion of the USDA nutrient composition database to include flavonoids has allowed us to update the classic Nutrient Rich Foods (NRF) model. The addition of flavonoids along the existing fruit-forward nutrients put berries on top.

This research was sponsored by the National Berry Crops Initiative.





Tuesday, May 7th - Scientific Presentations

Berries & the Brain



Dr. Barbara Shukitt-Hale
USDA & Tufts University

Session Chair

Current Research Review

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

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

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



Dr. Claire Williams
University of Reading, UK

Professor Williams is Chair of Neuroscience in the School of Psychology & Clinical Language Sciences at the University of Reading, UK. She received her PhD in Psychology from the University of Reading in 2000. Her research group, the Nutritional Psychology laboratory, investigates the health benefits of plant-derived chemicals. The main focus of her laboratory is the interplay between dietary intake and measures of psychological well-being such as cognitive performance, food preference, mood, and quality of life using a wide range of techniques (e.g. animal studies, randomised controlled trials, neuroimaging) and population groups (e.g., school-aged children, healthy adults, older adults, patients with mild cognitive impairment). The group have published a number of articles including a demonstration that improvements in spatial working memory induced by a high flavonoid 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. She has published more than fifty peer-reviewed research articles, four book chapters and four patents.



Dr. Claire Williams was unable to be with us today, Daniel Lamport, PhD, will be presenting in her stead.

Daniel Lamport, PhD
University of Reading, UK

Daniel Lamport, BSc, MSc, PhD CPsychol is currently a Lecturer in cognition and nutrition at the University of Reading, School of Psychology, UK. Daniel's research interests include the effect of diet, nutritional interventions, and metabolic disorders on neuropsychological function, and prevention of neurodegenerative disease.

Daniel completed his PhD in 2010 at the University of Leeds examining the effects of carbohydrate quality on cognitive performance in type 2 diabetes, before undertaking postdoctoral positions at Leeds and Reading. Most recently, his diverse research portfolio has included collaborations with Public Health England, UK research councils and industry sponsors, and he was invited keynote speaker at the 2017 International Conference on Polyphenols and Health.

The Chronic Effects of a Wild Blueberry Intervention on Cognition and Urinary Metabolites in 7-10 Year Old Children

Authors: Claire M. Williams¹, Katie L. Barfoot¹, Geoffrey Ista², Rodrigo Feliciano³, Daniel J. Lamport¹, Patricia M. Riddell¹ & Ana Rodriguez-Mateos²

Affiliations:

1. School of Psychology & Clinical Language Sciences, University of Reading, Reading, UK
2. Department of Nutritional Sciences, School of Life Course Sciences, King's College London, UK
3. Division of Cardiology, Pulmonology, and Vascular Medicine, University Dusseldorf, Germany

Consumption of a flavonoid-rich wild blueberry (WB) drink has been shown to improve episodic memory (EM), executive function (EF) and positive mood 2-6 hours post-consumption in 7-10 year olds (Whyte & Williams, 2015; Whyte et al, 2016; 2017; Barfoot et al, 2018). However, further investigation of those aspects of cognition and mood susceptible to repeated WB supplementation, alongside measurement of physiological mechanisms, are urgently required. Here we examined EF, EM and mood, alongside assessment of the changes in flavonoid urinary metabolites, following 4 weeks treatment with WB. Healthy, 7-10 year old children consumed either a 200ml WB drink (containing 253mg anthocyanins) or a 200ml matched placebo drink, daily for 4 weeks, according to a randomised, single-blind, between-groups design. EF (Modified Attention Network Task; MANT), Verbal memory (Auditory Verbal Learning Task; AVLT), mood (Child Positive and Negative Affect Scale; PANAS-C) and 24 hour urinary metabolites were assessed at baseline, 2 weeks and 4 weeks. Although no significant changes in mood or AVLT performance were seen, on the MANT, faster reaction times on incongruent trials, alongside maintenance of accuracy performance, was observed at 4 weeks relative to baseline for children treated with the WB intervention only. In terms of urinary output, as expected, increased excretion of polyphenols was seen for those children treated with WB when compared to baseline excretion. Changes in individual metabolites were also seen with significantly higher hippuric acid and benzoic acid observed in WBB participants' 24 h urinary excretion compared to placebo participants at the 2 week time point, although this was no longer evident at the 4 week timepoint. In contrast, significantly higher excretion of 3-(4-hydroxyphenyl) propionic acid and isoferulic acid 3-O- β -D-glucuronide was detected at 4 weeks for placebo participants compared to WBB treatment. To assess whether metabolite excretion was associated with cognitive performance at each visit, correlational analyses were performed on metabolite factors and composite EF and verbal memory scores. Here, increases in vanillic and ferulic acids were shown to positively correlate with increases in cognitive interference perhaps suggestive of an improved ability to encode primary information. Overall, this study revealed beneficial effects of chronic WBB consumption on executive function and attention in healthy 7-10 year olds after a 4 week chronic intervention alongside increases in total urinary polyphenol excretion for those treated with WBB compared to placebo. Some changes in individual metabolites were found across the 4-week intervention trial, and how these changes relate to better cognitive function will be discussed.

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

References:

1. Barfoot KL, May G, Lamport DJ, Ricketts J, Riddell PM & Williams CM (2018). The effects of acute wild blueberry supplementation on the cognition of 7-10-year-old schoolchildren. *European Journal of Nutrition*, DOI: 10.1007/s00394-018-1843-6.
2. Whyte AR, Schafer GW & Williams CM (2017). The effect of cognitive demand on performance of an executive function task following wild blueberry supplementation in 7 to 10 years old children. *Food and Function*, 8, 4129 - 4138.
3. Whyte AR, Schafer GS & Williams CM (2016). Cognitive Effects Following Acute Blueberry Anthocyanin Supplementation on 7 - 10 Year Old Children. *European Journal of Nutrition*, 55(6): 2151-2162.
4. Whyte AR & Williams CM (2015). A pilot study investigating the effects of a single dose of a flavonoid-rich blueberry drink on memory in 8-10 year old children. *Nutrition*, 31: 531-534



Dr. Chris Gill
University of Ulster

Chris Gill is Senior lecturer in nutrition, Associate Research Director for Biomedical Sciences at the Ulster University and thematic leader for “Phytochemicals and gut health” within the Nutrition Innovation Centre for Food and Health (NICHE). His research focuses on the influence of diet (both terrestrial and marine plants) on gut health investigated through in vitro, animal and human models. Dr Gill has published 30+ research papers, 6 book chapters and 1 patent, developed of an extensive research network with internationally leading research centres and secured significant research income from a range of sources from prestigious sources including the EU, BBSRC NPRC and FIRM. He is currently an Editor for the European Journal of Nutrition, a member of the ILSI Europe Human Microbiome Research Study Guidance Expert Group and a recent recipient of the Ulster University Distinguished Research Fellowship .

Impact of Raspberries on Vascular Architecture and Cognitive Function in a Transgenic Model of Alzheimer's Disease

Authors: Gill CIR¹, Torrens A¹, Mitchell CA¹, McDougall G².

Affiliations:

1. Nutrition Innovation Centre for Food and Health, Centre for Molecular Biosciences, Ulster University, Cromore Road, Coleraine, N. Ireland, UK
2. Environmental and Biochemical Sciences Group, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland.

Background: Disruption of microvascular architecture is a common pathogenic mechanism in the progression of numerous chronic diseases including Alzheimer's disease (AD) (1-3). A direct relationship links microvascular pathology and cognitive decline (1,4). Berries have been shown to improve cognition in animal models of AD, the effects of which cannot be fully explained by modulation of either neurogenesis or inflammation (5-7). Given the anti-angiogenic activity of berry (poly)phenols (8), we sought to determine if long-term feeding of raspberry (poly)phenols ameliorates cerebral microvascular pathology and improves cognition in previous studies by our group we showed that a drug re-purposed from the treatment of type 2 diabetics - Liraglutide (a GLP-1 agonist) restores cerebral microvascular pathology and improves cognition in the murine APP^{swe}/PS1^{E9} (APP/PS-1) transgenic model of AD (9).

Red raspberries (Glen Ample) and a yellow raspberry variety (Hutton breeding line 08108H1) were grown at the James Hutton Institute (Scotland, UK) in 2016, picked when ripe and frozen. Freeze dried (FD) powders were prepared. The red raspberry powders had higher total phenol content due mainly to their higher anthocyanin levels (which were absent in the yellow raspberry), although other minor qualitative differences in composition were noted by liquid chromatography-mass spectrometry (LC-MSn) profiling. We then conducted a 24 week feeding study in Male C57BL/6 mice (50 wild type, 50 APP/PS-1) aged 4 months, fed a normal chow diet enriched with either 100 mg/day glucose (control diet) or supplemented with FD powder (100 mg/ day) from either red or yellow raspberry fruit.

An untargeted approach using LC-MSn (10) coupled to an Orbitrap MS in both positive and negative ionisation modes was used to investigate the metabolic profiles of the brain and plasma samples of mice following 24 weeks on control diet, red or yellow raspberry feeding regimes. The MS data from the control and raspberry fed animals were compared and deconvoluted using XCMS software and mass spectral features that were consistently increased after raspberry feeding were identified after statistical analysis using PCA and validated OPLS-DA approaches. Fragmentation data and formula predicted from exact mass data allowed putative identification of several metabolites that increased over the feeding period. These included consistent changes in endogenous metabolites such as amino acid derivatives and lipids but also various components which appear to arise from the raspberry supplementation.

Ultrastructural changes in the morphology of surface cerebral microvascular architecture was assessed in a number of mice from each treatment group by induction of non-recoverable anaesthesia followed by trans-cardiac perfusion with successive solutions of isotonic saline, 2.5% glutaraldehyde and finally vascular casting using Pu4ii resin and visualisation by scanning electron microscopy. Cerebral microvascular architecture in wild-type mice was characterised by regularly spaced capillaries with uniform diameters, smooth surface morphology and clear inter-endothelial boundaries. In contrast, capillaries from APP/PS-1 transgenic mice were occasionally clustered (a sign of recent angiogenesis), had non-uniform diameters and showed evidence of inter-endothelial resin leakage, show disturbed microvascular architecture consistent with findings from previous studies. Despite detailed examination of vascular casts from APP/PS-1 mice treated with either yellow or red raspberry we were unable to detect any significant changes in microvascular architecture in these animals compared to those on the control diet.

Cognitive performance was assessed at baseline and 24 weeks post feeding by Open Field Test (OFT), Object recognition test (ORT) and Morris Water Maze (MWM). No significant differences were observed in the cognitive behaviour of wild type or APP/PS-1 mice across any of the dietary regimes at baseline. Analysis of cognitive differences after 24 weeks feeding indicated few significant changes in cognitive behaviour of wild type or APP/PS-1 mice across any of the dietary regimes in either ORT or OFT or MWM.

To conclude, consumption of red and yellow raspberries produced consistent changes in endogenous metabolites in both brain and plasma of wild type and APP/PS-1 mice. However, few effects on microvascular architecture or cognition were observed.

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

References:

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Dr. David Vauzour
University of East Anglia

Dr. David Vauzour received his PhD from the Faculty of Pharmacy, University of Montpellier (France) in 2004. His research over the last 14 years, based at the University of Reading (2005-2011), and at the Norwich Medical School, University of East Anglia, UK (2011- present) has focused on investigating the molecular mechanisms that underlie the positive correlation between the consumption of diets rich in fruits and vegetables and a decreased risk of (neuro) degenerative disorders and to develop novel dietary strategies to delay brain ageing, cognitive decline and cardiovascular disease, including stroke and vascular dementia. In this context, his initial work provided considerable insights into the potential for natural products to promote human vascular function, decrease (neuro)inflammation, enhance memory, learning and neuro-cognitive performances and to slow the progression of Alzheimer's and Parkinson's diseases. In particular, his major contribution has been to show that phytochemicals and in particular flavonoids exert such diverse biological effects through their modulation of intracellular signalling pathways (MAP kinase, PI3 kinase/Akt and Keap-1/Nrf2), biomarkers of cellular plasticity and resilience to exogenous stimuli. His recent interests concern how food bioactives modulate ApoE genotype-induced cardiovascular risk and neurodegenerative disorders and their underlying mechanisms. To date Dr Vauzour has published over 80 peer reviewed articles and currently serves as the Associate Editor for the journals "Nutrition and Healthy Aging" and Antioxidants. In addition, he is a member of the editorial board of "Nature Scientific Reports (Neuroscience)" and "Peer J (Pharmacology)" and is currently the co-the Chair of the ILSI Europe "Nutrition and Mental Performance Task Force".

Impact of Berry Polyphenols on the Gut-Brain Axis

Tremendous progress has been made in characterizing the bidirectional interactions between the central nervous system and the gastrointestinal tract [1]. This concept of a microbiome-gut-brain axis suggests that modulation of the gut microbiota is a tractable approach for developing novel strategies for the regulation of overall brain function [2]. This is particularly relevant for an ageing population for which cognitive decline is a common symptom and can be a harbinger of the development of neurodegenerative conditions, such as dementia.

The wide variation in the gut microbiota between individuals is a result of modulations of many genetic, environmental and physiological factors though changes in dietary composition and diversity are considered the main drivers of the shifts in microbiome structure and activity [3]. Such findings argue in favour of an approach of modulating the microbiome and indirectly brain functions with dietary interventions containing defined nutrients and food bioactives designed to promote healthier ageing. Amongst those nutrients, polyphenols have been consistently reported to play a protective role against cognitive decline [4] and have the ability to modify the microbiome composition and metabolism [5]. Emerging evidence including studies in animal models [6] and a recent prospective clinical trial [7] showed that cranberries had an impact on gut microbiota. However, the impact of cranberries in modulating cognitive functions through the gut-brain axis has never been investigated. Thus, the purpose of this presentation is to provide an overview of the gut-brain axis regulation, to discuss the regulation of cognitive functions and the gut microbiota by dietary polyphenols and to update on our recent intervention study aiming at investigating the impact of cranberries on the gut-brain axis. Potential molecular mechanisms of actions will also be discussed.

Keywords: Microbiome, polyphenols, cognition, cranberries, gut-brain axis

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Louise Dye, PhD
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Louise Dye is Professor of Nutrition and Behaviour and leads the Nutrition and Behaviour Group, in the Human Appetite Research Unit in the School of Psychology, University of Leeds. Louise is Academic Lead for the University of Leeds of the HEFCE catalyst funded N8 Agrifood Programme. The N8 Agrifood Programme brings together expertise across the 8 research intensive universities in the North of England in agriculture, food production and supply in a changing environment with a global reach. In her N8 role, Louise is interested in how to encourage and sustain dietary behaviour change at individual, organisational and societal levels, linking to global issues of food production/supply, inequality and health.

She is a member of the BBSRC Strategy Board for Biosciences for Health and of BBSRC's Diet and Health Research Industry club (DRINC) Steering Group. She has held MRC and Royal Society Postdoctoral Fellowships in the UK and Europe and an EU funded Marie Curie Professorial Fellowship in Jena, Germany. Professor Dye is a Chartered Health Psychologist and British Psychological Society member. She began her career in Human Psychopharmacology and has over 30 years' experience in the assessment of nutritional and pharmacological intervention on cognitive function and wellbeing. She is Associate Editor of Nutritional Neuroscience and the European Journal of Nutrition and Chair of the Scientific Advisory Board of the FP7 project "PREVIEW" which is aimed at the prevention of type 2 diabetes. Her research interests include functional foods for wellbeing, stress management, mental health and cognitive performance/decline and in altered metabolic states such as obesity and type 2 diabetes as well as genetic disorders such as PKU and Cystic Fibrosis. She is interested in the modification of glycaemic response by diet and the impact of food on stress and wellbeing, and has developed an experimental technique to permit repeated stress induction. Louise has published influential systematic reviews of the effects of breakfast on cognitive and academic performance and the effects of polyphenols on cognition. Her work on polyphenols includes a recent study of Concord grape juice which demonstrated enduring effects of Concord grape on cognitive and driving performance. She is a member of the Scientific Advisory Board of ILSI Europe and has served on 5 of their expert groups including Natural (plant based) Ingredients and Cognitive Function.

The Effects of Berry Polyphenols on Cognitive Function in Adults in the Context of Other Plant Based Ingredients: An ILSI Europe Systematic Review

There is increasing interest in identifying natural products that can support the improvement of mental performance and/or prevent age-related cognitive decline. There is a wealth of published work supporting the cognitive benefits of specific plant-based compounds. However, a systematic review of the evidence for cognitive effects of plant-based ingredients from studies, which apply rigorous methodology, their mechanisms of action and the potential synergy between ingredients is lacking.

ILSI Europe has brought together experts in the field to form a Nutrition and Mental Performance expert group to assess 'plant-based ingredients and cognitive performance'. The expert group set out to summarise and evaluate the existing literature relating to key plant-based ingredients, alone and in combination, in order to critically evaluate the evidence for benefits to cognitive performance. The objective of this systematic review is to highlight the most effective ingredients and combinations thereof and to provide guidance on methodological considerations that may reduce inconsistencies in future research.

Nine plant based ingredient groups were included. These were amino acids including taurine and L-theanine, botanicals such as ginkgo biloba, ginseng, sage, lemon balm, wild oat extracts, acetyl L-carnitine, hypersine, vinpocetine, bacopa, pycnogenol, ashwagandha, curcumin and alpha-lipoic acid, carotenoids, vitamins and minerals including Vitamin B, C and E and combinations thereof, phospholipids, omega 3 fatty acids, prebiotics and finally, polyphenols.

Inclusion criteria were applied such that studies that recruited healthy adults (age ≥ 20) of either sex, including pregnant women where the cognitive effects on their children (not preterm) were analysed, were included. Studies conducted in 3rd world countries (e.g. undernourished people or at risk for undernourishment) were also eligible to be included providing participants had no diagnosed disease. Furthermore, randomised controlled trials (RCT) of any duration (acute or chronic) that administered a plant-based ingredient of interest, delivered in any form e.g. liquid, gel capsule were included. For crossover studies, there was no restriction on the inclusion or length of wash-out period. Where a placebo was supplied with an additional ingredient, this same ingredient must have been administered with the active supplement. Follow-up studies following the intervention were also included. For parallel group studies to be included, randomisation to condition had to be stated within the article. However, for crossover trials, randomisation to treatment sequence was assumed to have taken place and therefore was not required to be stated explicitly within the article. Studies which included at least one standardised measure of cognitive performance for any cognitive domain were included. This could be either a primary or secondary endpoint. Studies that utilised a qualitative or subjective report as a measure of cognitive performance or assessed change in mood in the absence of an objective measure of cognitive performance were excluded. Five per cent of all studies per ingredient type returned by the search were independently assessed for eligibility. Each study was assessed for quality using the Cochrane Collaboration's tool for assessing risk of bias in randomised trials (Higgins et al., 2011). Risk of bias was performed by the original reviewer but confirmed by another, randomly allocated, reviewer. Embase (Embase Classic + Embase 1947) and PubMed electronic databases were searched with a final end date for inclusion of 10th July 2018.

With respect to polyphenols, the searches identified 16 studies of isoflavones and 12 studies of cocoa flavonols which met the inclusion criteria. Six studies of stilbenes (resveratrol) and 6 studies of nitrates (primarily beetroot) were also identified. There were 3 studies of flavanones which examined effects of orange juice.

In summary for polyphenols and berries in particular, the published literature included more reviews than well-controlled data papers. Few studies met the strict inclusion criteria, in part because the majority of studies were conducted in children (4 examined blueberries) or older adults with MCI (2 with blueberries, 1 with Concord Grape Juice, and 1 examined Cherry Juice). Effects of blueberries in older adults were examined in 4 included studies and examined alone or in combination with fish oil in 1 study. There were 3 studies of Concord grape juice (CGJ) included (2 acute and 1 chronic) and 1 CGJ study in older adults with MCI excluded. Two studies of blackcurrant juice, one of haskap berry and one study of plum juice were included. One study of cherry juice in older adults with dementia was excluded as was the only study of pomegranate juice which examined cognitive function postoperatively. The various studies did not show consistent effects on cognitive domains but varied in dose and duration. Those areas with consistent effects are discussed and common mechanisms considered. There are far fewer studies of berry anthocyanin based interventions than for other polyphenols and less than for other plant based ingredients such as micronutrients, amino acids and even ingredients such as L-theanine. This underlines the importance of further exploration of the potential benefits of berry polyphenols for cognitive function in carefully controlled randomised controlled designs with appropriate measures of cognitive function and appropriate methods of analysis applied.

Keywords: Cognition, flavonoids, memory, healthy adults, attention, executive function.

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Wednesday, May 8th - Scientific Presentations



Berries & Cardio-Metabolic Health



Dr. Britt Burton-Freeman
Illinois Institute of Technology

Session Chair

Current Research Review

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

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

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

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

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



Meijun Zhu, PhD
Washington State University

Dr. Meijun Zhu is an Associate Professor in the School of Food Science at Washington State University. She earned her BS and MS in Biochemistry at China Agricultural University, and her PhD in Food Microbiology from Iowa State University. She worked as a Postdoctoral Research Associate in Animal Physiology at University of Wyoming for three years. She joined WSU as an Assistant Professor in 2012 and was promoted to an Associate Professor in 2015. Her research focuses on molecular nutrition, particularly on impacts of food bioactive compounds on gut microbiota, intestinal epithelial health and metabolic disorders. She has published more than 190 peer-reviewed papers with H-index at 45. Her program has been well funded by USDA, NIH and commodity groups.

Dietary Raspberry Induces Browning of White Adipose Tissue via AMPK

Obesity is characterized with excessive lipid accumulation in white adipose tissue (WAT), which leads to chronic inflammation and metabolic dysfunction. Due to the presence of uncoupling protein-1 (UCP-1) and abundant mitochondria, brown adipose tissue is highly effective in burning lipids and glucose, suppressing adipose inflammation and metabolic dysfunction [1, 2]. Beige adipocytes are brown-like adipocytes distributed inside WAT, which are inducible, in a process referred to as WAT browning; inducing WAT browning is highly effective in preventing obesity and metabolic symptoms as evidenced by epidemiological, clinical and animal studies. Recent studies show that polyphenolics such as resveratrol can activate AMP-activated protein kinase (AMPK), a master regulator of energy metabolism, which enhances brown/beige adipogenesis [3]. Red raspberry contains abundant polyphenols, which are mainly anthocyanins and ellagitannins, which have high bioavailability. To test the effectiveness of raspberry in preventing obesity and metabolic dysfunction induced by dietary raspberry, we conducted mouse studies using the diet-induced obesity mouse model, where mice were challenged with a high fat and high sucrose diet mimicking the popular diets in western societies. We discovered that dietary raspberry at 5% of dry feed weight is effective in preventing obesity and metabolic dysfunction induced by high fat diet (HFD)[4]. The HFD feeding inhibited AMPK while raspberry supplementation activates AMPK, which correlated with the formation of brown/beige adipocytes [5]. To test the mediatory roles of AMPK, we used AMPK knockout mice, where the beneficial effects of raspberry in obesity and metabolic symptoms were largely abolished, showing that AMPK is essential in mediating the browning of WAT and preventing obesity due to raspberry supplementation [6]. Furthermore, *in vivo* BAT transplantation and *in vitro* cell culture studies showed that AMPK is essential for brown/beige adipogenesis (5). In summary, raspberry consumption is protective against obesity and metabolic dysfunction associated with consumption of common high energy western diet, and AMPK is a critical mediator for the beneficial effects of raspberry through inducing WAT browning.

Keywords: AMPK, Browning, Adipose tissue, Obesity, Metabolic syndrome, Raspberry

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Dr. André Marette
Laval University

Dr. Marette is Professor of Medicine at the Heart and Lung Institute, Laval Hospital, and Scientific Director of the Institute of Nutrition and Functional Foods at Laval University. He holds a research Chair on the pathogenesis of insulin resistance and cardiovascular diseases (CVD). Dr. Marette is an international renowned expert on the pathogenesis of insulin resistance and cardiometabolic diseases and his research has advanced the understanding of the physiological and molecular mechanisms of inflammation, and opened new possibilities for prevention and treatment and type 2 diabetes and CVD. He is also studying how nutrition and food ingredients can modulate the gut microbiota to protect against obesity-linked intestinal inflammation, fatty liver disease and type 2 diabetes. He has published over 220 papers, reviews and book chapters and was invited to give more than a hundred lectures at various national & international conferences in the last 10 years. He currently serves as Editor-in-Chief for the *Am J Physiol: Endo & Metab.* and has authored two books in the last few years, one entitled *La Vérité sur le Sucre* edited by VLB and one entitled *“Yogurt: Roles in Nutrition and Impacts on Health”*, edited by CRC press. Dr. Marette has received several awards for his work including the prestigious Charles Best Award and Lectureship from the University of Toronto for his overall contribution to the advancement of scientific knowledge in the field of diabetes.

Blueberry Anthocyanins and Proanthocyanins Improve Insulin Sensitivity in Diet-Induced Obese Mice Through Their Impact on the Gut Microbiota

Previous studies from our group and others have reported that polyphenol-rich extracts from various sources can prevent obesity and associated inflammatory and metabolic disorders in diet-induced obese mice and human subjects (1-7). However, the mechanisms underlying the beneficial effects of berry polyphenols remains poorly understood.

In the present study, we aimed to evaluate the effect of high-bush (HB) blueberry intake in a mouse model of obesity and associated metabolic diseases. This study was also designed to test whether anthocyanins (ANT) or proanthocyanins (PAC) found in HB blueberry could be involved in the modulation of body weight gain and metabolic homeostasis. We also aimed to demonstrate the role of the gut microbiota in the potential beneficial effects of HB blueberry extracts by using fecal microbiota transplantation (FMT). Our data show that both ANT and PAC extracts of HB blueberries improve insulin sensitivity in mice fed a high-fat and high sucrose (HFHS) diet promoting obesity. PAC-treated mice also showed increased leanness which was linked to greater physical activity. FMT studies further revealed that the improvement of insulin sensitivity by both HB blueberry ANT and PAC are linked to the modulation of the gut microbiota in HFHS-fed mice.

Keywords: Blueberry polyphenols, Microbiome, Microbiota, Obesity, Insulin resistance, Energy metabolism, Glucose metabolism

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Dr. Rodriguez-Mateos is an Assistant Professor at the Department of Nutritional Sciences, Faculty of Life Sciences and Medicine of King's College London. She received her PhD and conducted her postdoctoral studies at the Department of Food and Nutritional Sciences of the University of Reading, UK. She then worked as a Research Group Leader in Diet and Cardiovascular Health at the Division of Cardiology and Vascular Medicine of the University of Dusseldorf in Germany, before joining King's College in 2016. Her research aims to investigate the health benefits of plant food bioactives, with a strong focus on understanding the bioavailability, metabolism and cardiovascular health benefits of polyphenols. Her expertise includes development and validation of analytical methods for identification and quantification of polyphenols in foods and biological samples and performance of randomized controlled trials with cardiovascular outcomes. She has obtained funding from the UK Research Councils, European Union, charities and industry. Dr Rodriguez-Mateos is an Associate Editor for the journal *Food and Function* and a member of the editorial board of *Nutrition and Healthy aging*. She is a Registered Nutritionist, fellow of the UK Higher Education Academy, and member of the Royal Society of Chemistry, Nutrition Society and American Society for Nutrition.

Berry Polyphenols and Endothelial function: Findings From Human Studies and Mechanistic Insights

Berries are getting increased attention due to their potential cardiovascular health benefits. To give adequate dietary recommendations to the general public regarding the consumption of berries, it is important to understand which bioactives in berries are responsible for the effects and the mechanisms of action in the vascular system. To address this, a number of randomized controlled human intervention studies were conducted in parallel with extensive targeted metabolomics. Acute consumption of wild blueberries, raspberries, cranberries, and aronia berries improved endothelial function, measured as flow-mediated dilation (FMD), by 1 to 2.5 % in healthy men (1-6, unpublished data). Daily consumption of wild blueberries, cranberries and aronia berries for 4 to 12 weeks led to improvements in FMD of the same magnitude (1, 6, unpublished data). Furthermore, berry (poly)phenols were bioavailable in plasma and urine and a number of metabolites were significantly associated with acute and chronic increases in FMD (1-10, unpublished data). Studies with purified anthocyanins suggest that they are major contributors to the bioactivity of berries but cannot explain the whole effect, while proanthocyanidins do not seem to have a significant effect on vascular function (1,11). Dose-response studies indicated that effects on endothelial function were significant at dietary achievable amounts and that effects plateau when high amounts were given (1,2,5). (Poly)phenol metabolites injected in vivo in mice demonstrated bioactivity and human nutrigenomic analysis revealed new molecular targets that may be underlying the health benefits of berries (1). In conclusion, berry (poly)phenols are bioavailable, and their circulating metabolites improve endothelial function in healthy subjects modulating important gene programs. Our data support the efficacy of berries for the primary prevention of cardiovascular disease.

Key Words: Berry polyphenols, endothelial function, cardiovascular health , bioavailability, randomised controlled trials

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Dr. Sarah A. Johnson
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Sarah A. Johnson, PhD, RDN is an Assistant Professor and Director of the Functional Foods & Human Health Laboratory in the Department of Food Science and Human Nutrition at Colorado State University. Her research program aims to integrate multiple disciplines including nutrition, food, agricultural, and biomedical sciences to perform translational research studies focused on critically examining the efficacy and mechanisms by which functional foods, namely flavonoid-rich berries, improve cardiovascular disease risk factors and attenuate vascular dysfunction in high-risk aging populations. Current work focuses on clinical efficacy of blueberries and aronia berries to attenuate age-related vascular dysfunction, and underlying mechanisms responsible for clinical efficacy and physiological effects in general.

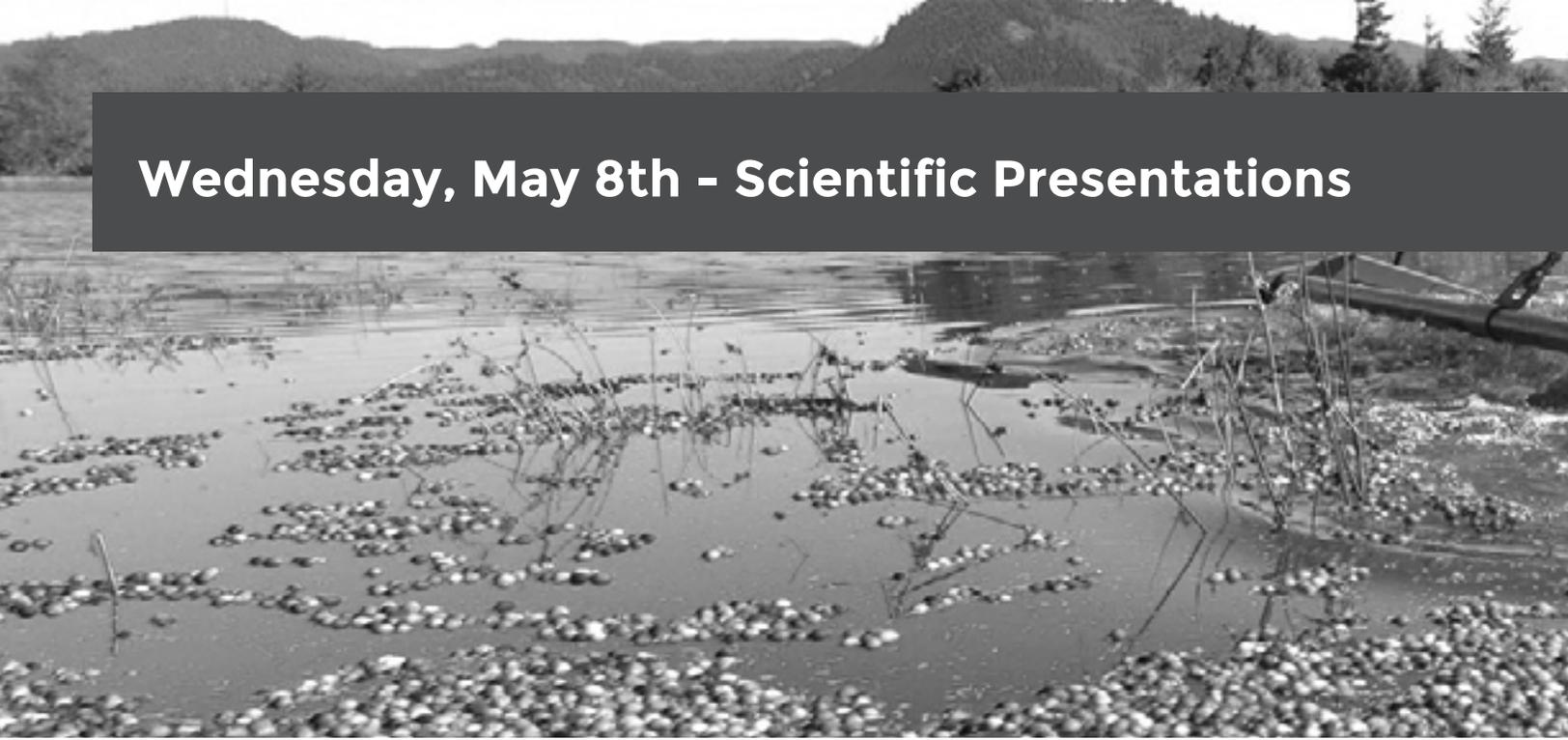
Dr. Johnson has received honors and awards such as the Emerging Leaders Network Award from the Institute of Food Technologists, the Abbott Nutrition Award in Women's Health from the Academy, the Junior Faculty Author Award from the Research Dietetic Practice Group of the Academy of Nutrition and Dietetics, and the Clinical Emerging Leader Award from the Medical Nutrition Council of the American Society for Nutrition. She is on the Editorial Board for the Journal of Medicinal Food. She is a Member of the Council on Research and Past Chair of the Evidence-Based Practice Committee with the Academy of Nutrition and Dietetics.

Blueberries for Attenuating Age-Related Vascular Dysfunction: Evidence and Opportunities

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. Aging is the primary risk factor for CVD, in large part due to adverse modifications to the vasculature, particularly the arteries. During aging, adverse structural and functional changes in the arteries lead to the development of vascular dysfunction, namely vascular endothelial dysfunction and arterial stiffness. Age-related vascular dysfunction contributes to the development of atherosclerosis and hypertension (isolated systolic hypertension in particular), and can lead to stroke, myocardial infarction, and other adverse events including death. Mechanisms underlying vascular dysfunction with aging are complex and multifactorial, but reduced nitric oxide (NO) bioavailability secondary to excessive superoxide-driven oxidative stress and chronic inflammation is believed to be a central contributor. Numerous factors such as menopause, obesity, and metabolic syndrome can accelerate these processes and therefore vascular dysfunction in aging individuals, or cause premature development in others. Elevated blood pressure and hypertension (HTN) further perpetuate these processes leading to greater impairments in vascular function. Importantly, greater than two-thirds of the United States population has elevated blood pressure or stage 1-HTN.

Previous research with blueberries strongly suggests their potential for improving blood pressure, attenuating vascular dysfunction, and therefore reducing age-related CVD risk. Blueberries are rich in bioactive compounds including flavonoids, phenolic acids, and pterostilbene. These compounds, and derivatives resulting from gut microbial and phase II metabolism, are known to attenuate oxidative stress and inflammation. We previously demonstrated that consumption of 22 g/day freeze-dried blueberry powder (equivalent to ~1 cup fresh blueberries) for 8 weeks reduced blood pressure and arterial stiffness and increased circulating NO metabolites in postmenopausal women with pre- and stage 1-HTN. However, mechanisms contributing to these effects remain unknown. Several other clinical trials have demonstrated improvements in vascular function and/or blood pressure, though findings have not been consistent likely due in part to baseline health status. Preclinical research has provided important insight into potential mechanisms of action. Altogether, previous research has laid important groundwork for current and future research investigating the impact of blueberry consumption on age-related vascular dysfunction.

Our laboratory is currently examining the efficacy of consuming 22 g/day of freeze-dried blueberry powder to improve endothelial function in postmenopausal women with elevated blood pressure or stage 1-HTN and baseline vascular endothelial dysfunction in a 12-week randomized, double-blind, placebo-controlled, parallel arm clinical trial. Assessments being performed will provide important mechanistic insight into the extent to which blueberries improve vascular endothelial function through reductions in oxidative stress and inflammation. We will also begin exploring the impact of blueberries (at the same dose) on blood pressure and vascular function in middle-aged/older men with elevated blood pressure or stage 1-HTN and baseline endothelial dysfunction in a 12-week randomized, double-blind, placebo-controlled, parallel arm clinical trial. At this relatively low dose, blueberries represent a practical, safe, well-accepted, and cost-effective therapy for the treatment of elevated blood pressure or HTN and vascular endothelial dysfunction in aging populations with a high-risk for developing CVD.



Wednesday, May 8th - Scientific Presentations

**Berry Phytochemicals and Metabolites
on Cardiovascular Function**



Mary Ann Lila, PhD
North Carolina State University

Session Chair

Current Research Review

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

Dr. Lila is currently a co-Director of an ambitious public-private Plant Pathways Elucidation Project (P2EP) which synergizes the talents of academia and industry. Other current research projects include a Foundation for Food and Agriculture Research initiative on 'Closing the gap in delivery of fruit and vegetable benefits', USDA-funded initiatives on polyphenol-protein colloids for attenuation of food allergies, on the science behind Alaska's traditional subsistence lifestyle, and on saffron as a high value culinary and medicinal niche crop, and a Comparative Medicine Institute grant for developing the neonatal pig as a model for human food allergies.

Dr. Lila has been awarded the Paul A. Funk Scholarship Recognition Award (the premier research award in the College of ACES, University of Illinois), the Spitz Professorial Career Excellence Award, the Faculty Award for Excellence in Research, the University Scholar Award, the Amoco Award for Excellence in Undergraduate Instruction, and the Lilly Endowment Teaching Fellowship. Dr. Lila has ongoing research projects in Australia, New Zealand, and multiple countries in Europe, the Americas and Africa, and is Vice President of the Global Institute for BioExploration (GIBEX). In 1999, Dr. Lila won a Fulbright Senior Scholarship to conduct research and outreach in New Zealand, and returns to Australasia at least once/year.



Carl Keen, PhD
University of California, Davis

Dr. Keen has been a member of the nutrition faculty at the University of California, Davis since 1981. He was the Chairman of the Department of Nutrition from 1993 to 2006, and he was the Interim Director of the University of California, Davis, Foods for Health Institute from 2006-2007. Dr. Keen has over 650 peer reviewed scientific publications. He has won several awards including the American Institute of Nutrition Bio-Serv Award (1985), and the American Institute of Nutrition Research Award (1995), and the McCormick Science Institute Research Award (2014). In 2002, he was recognized by the Institute of Scientific Information as a highly cited researcher in the Agricultural Sciences, and in 2004 he was recipient of the Teratology Society's Warkany Award for research accomplishments in developmental biology.

In 2006, he was appointed as the first holder of the Mars Family Endowed Chair in Developmental Nutrition. He has served on numerous government boards and panels, and editorial boards. Dr. Keen's current research is focused in two areas; the investigation of the influence of maternal diet on the risk for pregnancy complications (maternal and conceptus) and the influence of diet on the risk for age-related chronic diseases. Regarding the influence of diet on embryonic and fetal development, his research group has been studying the acute and long-term consequences of micronutrient deficiencies on embryonic and fetal development for almost three decades. The majority of their efforts has centered on the characterization of the effects of primary and secondary mineral deficiencies on the developing vascular system and brain. Regarding the influence of diet and chronic disease, for the last couple of decades, the laboratory group has focused on the study of dietary factors that influence the risk for vascular disease. This includes the cardiovascular health benefits associated with plant food rich diets that is attributed to their flavanol content.

Effects of Short-Term Consumption of Strawberry Powder on Parameters of Vascular Health in Adolescent Males

Authors: Carl L. Keen¹, Roberta R. Holt¹, Dragana Djurica¹, Emily Padhi¹, Carolyn M. Slupsky¹, and Robert M. Hackman¹

Affiliations:

1. Department of Nutrition, University of California, Davis

Our laboratory group and others have been building on studies on favorable effects of California strawberries on vascular health¹. Freeze dried strawberry powder (FDSP) contains a number of nutrients that may have direct beneficial effects on plasma lipids and vascular function. Strawberries are rich in flavonoids, particularly anthocyanins, flavanols, flavonols, ellagic acid and its glucose ester, ellagitannin. FDSP also contains a substantial amount of potassium, the increased dietary intake of which is known to positively affect blood pressure². Similarly, FDSP intake can be a substantial source of dietary nitrate, which can be converted to nitrite and subsequently vascular active nitric oxide (NO) in the gastrointestinal tract via reduction by polyphenols, heme-containing compounds, and certain bacteria. In addition, FDSP phytochemicals may affect gastrointestinal tract microbiota and associative metabolites that in turn can have secondary positive effects on the vascular system. For example, a reduction in Bacteroidetes compared to Firmicutes has been observed in obese compared to lean populations³, which can be rectified with fecal transplantation in mouse models⁴.

We have conducted a trial examining the effects of daily FDSP intake on cardiovascular health and gastrointestinal tract microbiome. Twenty-five overweight or obese adolescent males, ages 14-18 years, were enrolled into a randomized, double-blind, crossover study, and asked to consume 50g of FDSP or an isocaloric control powder, daily for 1 week. Before and after each test period, measures of microvascular function and total plasma nitrate/nitrite were collected as a fasting sample and 1 hour after FDSP intake. Plasma nitrate/nitrite levels, a marker of dietary nitrate intake and NO metabolism, significantly increased 1 hour after consuming the FDSP. A significant increase in short-term microvascular function was observed, which was dependent on circulating levels of total plasma nitrate/nitrite. Taken together, the data from this study supports the concept that strawberries can provide vascular health benefits to overweight and obese adolescent males. Of interest is that the observed effects were associated with an increase in the circulating levels of nitrate/nitrite. Important, as nitrite can be converted to NO through a number of pathways to include the microbiota. In an ongoing trial, we are assessing the influence of 4 weeks of daily FDSP intake on gut microbiota composition, microbial-derived metabolites, and associated changes with microvascular function, plasma nitrate/nitrite, and metabolism in overweight and obese postmenopausal women. The background and design of this current trial along with the results of Trial 1 will be presented, as well as, a discussion on the next steps towards further defining the positive vascular effects that can be associative with chronic strawberry feeding.

Keywords: adolescents, nitrate, vascular function

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Aedin Cassidy, PhD
University of East Anglia

Aedin Cassidy is Head of the Dept. of Nutrition & Preventive Medicine at Norwich Medical School, University of East Anglia. Her research focuses on understanding the impact of plant bioactives on cardiometabolic health with current research focusing on anthocyanins, the microbiome and cardiovascular health. She was awarded the Royal Society Wolfson Research Merit Award in 2013 and has served on various committees and expert panels including RAE2008 and REF2014. She is a Fellow of the Society for Biology and the Royal Society of Chemistry. She is a visiting scientist at the Harvard TH Chan School of Public Health.

The Impact of Blueberries on Cardiometabolic Health in Participants with Metabolic Syndrome – Results From a 6-Month Trial

Although anthocyanin intake is associated with reduced cardiovascular disease risk in prospective studies, few long-term randomised controlled trials (RCT) have been conducted in individuals at elevated risk. Following ingestion, anthocyanins are extensively metabolised (by Phase I & II metabolism and the gut microbiome) with the gut microbiome likely playing a key metabolic role, catabolising unabsorbed constituents into smaller molecules such as phenolic and aromatic acids, which are also absorbed (1,2). For anthocyanins consumed in the diet, the parent compounds may not be responsible for bioactivity; instead this may be mediated by metabolites present in the systemic circulation (2,3). Data from limited available trials show that following berry or anthocyanin intake there is extensive variability in metabolite levels. This wide inter-individual variability in metabolism (15-99% of the ingested intake recovered as a wide range of urinary metabolites (1, 3-4) suggest that metabolism may be critical in explaining the differential responses in cardiovascular risk biomarkers observed in clinical trials (responders v non-responders) (5-7).

We recently completed a clinical trial to examine the effect of 6-month blueberry intake on clinically-relevant biomarkers of insulin resistance, vascular function, lipid status and anthocyanin metabolism in participants with metabolic syndrome (MetS) (8). In this double-blind, parallel RCT (n=115), we fed two dietary achievable blueberry intakes (equivalent to 1/2 /1 cup/d, fed as 13 g v 26 g freeze-dried blueberries daily containing 182 and 364 mg/d anthocyanins). Insulin resistance was assessed via HOMA-IR (primary endpoint) and confirmed by [6-6-2H2] glucose-labelled, 2-step hyperinsulinemic clamp (n=20). Clinically-relevant cardiometabolic endpoints (including flow mediated dilatation (FMD), blood pressure (BP), augmentation index (AIx), pulse wave velocity (PWV)) and anthocyanin metabolism (in urine and blood) were also assessed. Shot-gun sequencing analyses of the gut microbiome is ongoing.

We found that 1 cup improved endothelial function (FMD): [+1.45%, 95%CI: 0.83, 2.1; p<0.001], AIx: [-2.24%, 95%CI: -3.97, -0.61; p=0.04] and cyclic guanosine monophosphate concentrations ((cGMP): [+0.99 pmol/mL, 95%CI: -9.24, 11.2; p=0.04]). In statin non-users (n=71), elevated HDL-C [+0.08mmol/L; p=0.03], HDL particle (HDL-P) density [+0.48 n, x10⁻⁶; p=0.002] and Apo-A1 (+0.05 g/L; p=0.01) levels were observed following intervention. Concurrently, LC-MS/MS analysis confirmed associations between increased downstream anthocyanin metabolite levels and changes in measures of vascular health. The intervention had no effect on insulin resistance (assessed via HOMA-IR (primary endpoint) and confirmed (in n=20) by [6-6-2H2] glucose-labelled, 2-step hyperinsulinemic clamp), PWV or BP. The 1/2 cup intake had no effect on any biomarkers in this high risk group.

Despite no change in insulin resistance, we show for the first time, clinically relevant changes in vascular function and underlying improvements in NO bioactivity and lipid status, following 1 cup/d of blueberries for 6-months. Blueberries should be included in dietary strategies aimed at improving individual and population heart health.

To understand the importance of metabolism, particularly microbiome-mediated biotransformation, in explaining the CV health effects of anthocyanins a combination of epidemiological studies and dietary intervention trials (acute and chronic) are needed. This will allow the identification of metabolites and microbial composition as intermediate biomarkers for dietary interventions and will be critical in identifying and validating biomarkers to employ to examine associations of bioavailable flavonoids with CV health in prospective studies. Ultimately, detecting the influence of anthocyanin intake on microbiota shifts and metabolism will strengthen the case for causality of anthocyanin-disease relationships.

Keywords: Blueberries, endothelial function, anthocyanins, gut microbiome, metabolic syndrome

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Dr. Arpita Basu
University of Nevada, Las Vegas

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 Kinesiology and Nutritional Sciences, UNLV.

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

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

Dietary Berries, Insulin Resistance and Diabetes

According to the CDC, more than 30 million Americans (9.4% of US population) were estimated to have diabetes mellitus (DM) in 2015. This number is expected to double or triple by the year 2050. Pharmacologic interventions are costly and are associated with adverse side effects, thus nutritional therapy continues to play an important role in the prevention, development and treatment of this disease. Among various foods and beverages high in dietary bioactive compounds, colorful berries have been shown to play an important role in the management of hyperglycemia and diabetes-related vascular complications. In our previous studies in participants with pre-diabetes or the metabolic syndrome, supplementation of freeze-dried blueberries and strawberries were shown to improve blood pressure and surrogate markers of vascular dysfunction, but with no significant changes in blood glucose and/or insulin resistance. On the other hand, we observed a significant decrease in postprandial hyperglycemia and inflammation, when dietary cranberries or red raspberries were administered along with a high-fat breakfast meal in participants with type 2 diabetes. These clinical observations are further supported by experimental data on the role of berry bioactive compounds in improving glucose metabolism and glycemic control in animal models of type 2 diabetes. This presentation will provide an overview of clinical studies on the effects of dietary berries on glycemic control, and results from our ongoing studies on the effects of strawberries on insulin resistance and diabetes-related 'transcriptomics'.

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Dr. Colin D. Kay
North Carolina State University

Dr. Colin Kay is an associate professor in the Department of Food, Bioprocessing and Nutrition Sciences at the North Carolina State University's Plants for Human Health Research Institute. Dr Kay's research is centered on establishing the metabolism of dietary phytochemicals and the potential impact this has on their biological activity. His research core is focused on the development of quantitative metabolomic MS/MS methodologies for establishing microbial-derived biosignatures of dietary phytochemical consumption.

Characterizing the Berry Metabolome: Insights & Examples from Bilberry, Blueberry and Strawberry Interventions

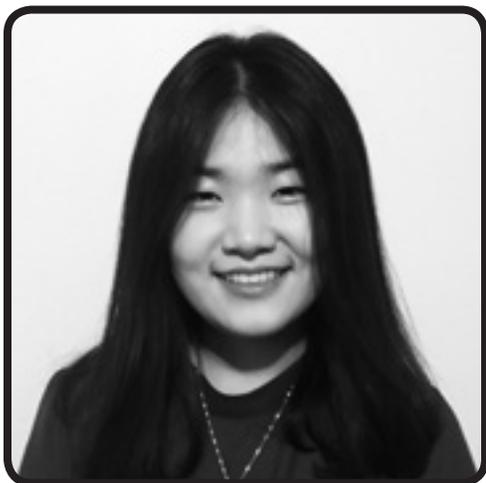
Introduction: Progress establishing biomarkers of intake of dietary phytochemicals has generally been sluggish and complicated by the recent realization of the significant contribution of the microbiome to the human metabolome.

Methodology: An MSn quantitative metabolomics database and analytical workflow was created to characterize the contribution of diet to the human metabolome, with focus on microbial metabolites of polyphenols. As online compositional databases lack comprehensive metabolome details, our data-capture workflow utilized a systematic review (using PubMed, Web of Knowledge and SCOPUS) aiming to capture compositional data relative to the “berry metabolome” (focusing initially on the most highly-consumed berries: blueberry and strawberry). The search returned 305 results. 174 titles and 41 abstracts did not meet inclusion criteria, leaving 90 articles for full text extraction of composition data. Over 1200 unique structures were identified in the search (“pre-clean”), however, greater than 900 of those were synonyms or reflected inaccurate nomenclature and were removed, leaving 300 identified structures. The number of major classes included alkaloids, anthocyanins, caffeoylquinic acid, condensed tannins, coumarins, flavanols, flavonols, hydrolyzed tannins, hydroxycinnamic acids, lignans, phenolic acids, phenols and stilbenes. After target identification, our workflow included compound fragment transition optimization via direct syringe infusion of pure reference standards following source and chromatography optimization using complex analyte mixtures comprising greater than 50 metabolites. Following compound and source optimization, the workflow included optimization of MRM, scheduled MRM (sMRM) and advanced scheduled MRM (ADsMRM) parameters, established sequentially using large pools of metabolites (100 - 150) spiked in pooled blank or synthetic serum/plasma or urine matrices and injected multiple times at multiple concentrations (LLOQ, MCQ, ULOQ) in succession to establish chromatography drift, signal stability, background noise and integration thresholds. The current berry metabolome database reflects approximately 200 analytes comprising over 100 reference standards and 100 putatively identified metabolites for which no reference standards exist. Putative targets were established using a series of injections of pooled clinical samples where MRM transitions are programmed for optimized base structures (i.e., unconjugated pre-metabolized analytes) and known biological conjugate masses, such as glucuronide, sulfate, glycine and methyl. The MRMs were run on pooled samples as a series of dilutions to identify linearity of signals (i.e., signals proportionally affected by overlapping precursor and product signals). The resulting database and MS/MS berry metabolome methodology has been utilized to interrogate over 1500 biospecimens to date, derived from studies feeding various polyphenol-rich diets, including interventions with blueberry, strawberry, bilberry and elderberry.

Results & Discussion: The metabolomes of the berry varieties studied were similar in their diversity of polyphenolic microbial metabolites (i.e., benzoic, phenylacetic, phenylpropanoic, cinnamic acids). This is not surprising considering the similarity in phytochemical makeup of commonly consumed berries (anthocyanidins, PACs, chlorogenic acids, ellagic acids) and diversity of microbial enzymes and pathways for aromatic compound catabolism. Individual metabolite structures were identified between 50 nM and 250 nM (SD ± 400 - 1500). The metabolites observed in highest abundance were consistent across studies, reflecting phenylacetic, hydroxybenzoic, hydroxy-methoxybenzoic and hippuric acids, hydroxyl-methoxybenzaldehyde and glucuronides of phenylacetic and phenylpropanoic acids. Following berry interventions feeding one to three portions of berries, these metabolites were found to reach considerable concentrations in some participants (1 - 40 µM), with significant diversity of glucuronide and sulfate isomers, generally found between 10 and 350 nM. Metabolites found in the highest abundance also displayed the greatest variability in concentration, which likely reflects microbiome diversity, study design and host intestinal transit. If structurally similar metabolites were to have additive or synergistic activities, there would be considerable potential to alter host physiology as their cumulative blood levels were observed at concentrations greater than 300 µM. Conclusion. Considering the structural similarity of polyphenol metabolites derived from a diversity of precursor phytochemicals, considerable dietary confounders should be considered when designing studies exploring the unique metabolome of berries, including coffee, tea, wine, potato, nuts, apples, stone fruit, and tropical fruits.

Keywords: Metabolome, Microbiome, Quantitative Metabolomics, (Poly)phenol

Wednesday, May 8th - Junior Investigators Oral Presentations



Inah Gu

PhD Student at the University of Arkansas

Inah is a PhD student in Food Science at University of Arkansas. She received her B.S. degree in Food Science and Nutrition from Pusan National University, South Korea, and her M.S. degree in Food Science from the University of Arkansas. She works with her advisor Dr. Sun-Ok Lee, focusing on how dietary phytochemicals and bioactive components in foods and natural extracts exert human health benefits. Inah's research aims to identify and fractionate bioactive components in different kind of berries and investigate the effects of berry bioactive compounds in chronic inflammation. She is a member of the American Society for Nutrition, and Korean-American Scientists and Engineers Association.

Berry phenolics and volatiles inhibit inflammatory cytokines secretion in LPS-stimulated RAW264.7 cells through suppression of NF- κ B signaling pathway

Authors: Inah Gu, Cindi Brownmiller, Luke R. Howard, Sun-Ok Lee

Affiliation: Department of Food Science, University of Arkansas

BACKGROUND: Berries are rich in minerals, vitamins, and especially phenolics and volatiles. Berry phenolics have received much attention due to their protective effect against many chronic diseases. However, there is limited information available on health-promoting activities of berry volatiles.

OBJECTIVE: The objective of this study was to investigate the anti-inflammatory effect of berry phenolics and volatiles using a lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cell model by measuring expression levels of key pro-inflammatory cytokines and the nuclear factor-kappa B (NF- κ B).

METHODS: RAW264.7 cells were pretreated with 3 different dilutions (diluted 50, 100, and 200-fold) of six berry (blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry) phenolic or volatile extracts for 1h. Cells were then incubated with or without LPS (100ng/ml) for 24h. The productions of nitric oxide (NO), and inflammation-related cytokines (IL-6 and TNF- α) were examined by Griess reaction and ELISA kits. Protein expression of key factor, NF- κ B was also assessed by Western blotting.

RESULTS: Berry phenolics and volatiles significantly inhibited the LPS-induced NO production by 38-93% and 47-79%, respectively ($p < 0.05$). There were no significant differences among three different dilutions of berry phenolics and volatiles in the RAW264.7 cells. Except black and red raspberry, berry volatiles significantly decreased the level of TNF- α and IL-6 in LPS-stimulated RAW264.7 cells ($p < 0.05$), comparable to berry phenolics. Moreover, berry phenolics and volatiles reduced the nuclear translocation of NF- κ B to prevent its activation by blocking the phosphorylation of p65 and inhibitor protein of NF- κ B (I κ B) compared with LPS-stimulated control.

CONCLUSIONS: These findings showed that berry volatiles from six common berries have comparable anti-inflammatory effects as berry phenolics through suppression of NO and cytokine expression via NF- κ B down-regulation, suggesting that berry volatiles may have the potential benefits as effective natural agents with anti-inflammatory activities to prevent inflammatory diseases.

KEYWORDS: Berry volatiles, Berry phenolics, Anti-inflammatory effect, Inflammatory cytokines, Nuclear factor-kappa B

Natalie VandenAkker
Doctoral Candidate at the University of Maine



Natalie is a current doctoral candidate in the School of Food and Agriculture at the University of Maine (2017-present), where she researches the role of red raspberries on vascular function and obesity-induced inflammation under the tutelage of Dr. Dorothy Klimis-Zacas. Natalie attended Worcester State University where she graduated in 2013 with a bachelor's degree in biology with minors in chemistry and business and in 2015 received a master's degree in biotechnology where she researched regenerated parsnip and carrot plants from cultured callus cells. Her professional experience in research began in 2014 at Sanofi-Genzyme a pharmaceutical company as an animal research technician and continued at TetraGenetics as a research associate where she gained experience with *Tetrahymena thermophila* to produce recombinant proteins.

She received the Food Science and Human Nutrition program's outstanding PhD Graduate Student Award (2018) and has served on the board of the Graduate Student Government at the University of Maine.

*Red raspberry (*Rubus idaeus*) consumption restores the impaired acetylcholine-induced vasorelaxation in the aorta of the obese Zucker rat, a model of the Metabolic Syndrome*

Authors: Natalie E. VandenAkker, Stefano Vendrame, and Dorothy Klimis-Zacas

Affiliation: School of Food and Agriculture, University of Maine

BACKGROUND: The Metabolic Syndrome (MetS) is associated with vascular dysfunction.

OBJECTIVE: The effect of whole red raspberry (WRR) consumption on vascular function was investigated in the obese Zucker rat (OZR), a model for the MetS.

METHODS: Male OZRs (n=16) and their lean littermates (LZR) (n=16) at 8 weeks of age, were placed on a control (C) or an 8% WRR-enriched diet for 8 weeks. Acetylcholine (Ach)-mediated vasorelaxation was studied in isolated aortic rings in the presence or absence of specific inhibitors L-N-monomethyl-arginine (L-NMMA) and mefenamic acid (MFA). Plasma NO levels, aortic effluent prostacyclin I₂ (PGI₂) and thromboxane A₂ (TXA₂) were measured. Gene expression of aortic inducible NOS (iNOS), endothelial NOS (eNOS) and cyclooxygenase-2 (COX-2) were evaluated.

RESULTS: OZR exhibited impaired relaxation to Ach compared to the LZR at 10⁻⁸ and 3x10⁻⁸M. Enhanced Ach relaxation was observed in the OZR-WRR at 10⁻⁸ and 3x10⁻⁸M. Following pre-treatment with L-NMMA, maximal vasorelaxation response was higher in the OZR-C compared to the LZR (80.37 ± 2.95% vs 60.65 ± 4.67%, p<0.0001). The maximal vasorelaxation response in OZR-WRR (70.99 ± 3.02%, p<0.05) was lower compared to the OZR-C in the presence of L-NMMA. Vessel sensitivity (pD₂) to Ach was higher in the OZR compared with LZR, in the absence of the inhibitors (7.63 ± 0.58 vs 7.28 ± 0.04, p<0.05) and following L-NMMA treatment (7.09 ± 0.11 vs 6.45 ± 0.16, p<0.05). PGI₂ concentration was higher in the OZR-C (9644.71 ± 1460.37pg/mg) compared to the LZR (5523.36 ± 359.29pg/mg, p<0.05). PGI₂ was attenuated (4905.91 ± 550.02pg/mg, p<0.01) in the OZR-WRR. Aortic expression of eNOS (p<0.01) and COX-2 (p<0.05) were downregulated in the OZR-WRR.

CONCLUSIONS: Whole Red Raspberry restores the impaired vasorelaxation of the OZR by enhancing the Ach-induced vasorelaxation response, increasing aortic effluent PGI₂ concentration and downregulating expression of eNOS and COX-2.

KEYWORDS: metabolic syndrome, red raspberries (*Rubus idaeus*), vascular function, vasorelaxation, nitric oxide synthase, cyclooxygenase-2, prostacyclin I₂, thromboxane A₂

Wednesday, May 8th - Junior Investigators Oral Presentations



Claudia Favari

PhD Student at the University of Parma

Claudia is a PhD student in Food Science at the Laboratory of Phytochemicals in Physiology, led by Prof. Daniele Del Rio within the Human Nutrition Unit of the Department of Food and Drugs and of Veterinary Medicine (University of Parma). Her research activity is presently focused on polyphenols and their microbiota-derived metabolites, and on the understanding of their role in human physiology.

Plasma levels of phenyl-gamma-valerolactones derived from cranberry flavan-3-ols: a dose-response study

Authors: Claudia Favari¹, Pedro Mena¹, Claudio Curti², Geoffrey Istas³, Christian Heiss⁴, Daniele Del Rio^{5,6}, Ana Rodriguez-Mateos³

Affiliation:

1. Human Nutrition Unit, Department of Food & Drug, University of Parma, Parma, Italy
2. Department of Food & Drug, University of Parma, Parma, Italy
3. Department of Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK
4. Department of Clinical and Experimental Medicine, Faculty of Health and Medical Science, University of Surrey, Guildford, UK
5. Department of Veterinary Science, University of Parma, Parma, Italy
6. School of Advanced Studies on Food and Nutrition, University of Parma, Parma, Italy

BACKGROUND: Cranberries are a rich source of poly(phenols), mainly monomeric and oligomeric flavan-3-ols. However, information on the appearance of their main circulating microbial metabolites, namely phenyl- γ -valerolactones, is lacking.

OBJECTIVE: The aim of the present study was to evaluate the absorption, metabolism and urinary excretion of cranberry flavan-3-ols through the targeted analysis of phenyl- γ -valerolactones and their related phenyl-valeric acids, after consumption of cranberry juice containing different amounts of total (poly)phenols (TP).

METHODS: A six-arm acute crossover, randomized, double-blinded, controlled intervention trial was performed in ten healthy males (Feliciano et al., *Nutrients*, 9(3), 268, 2017). Volunteers consumed a cranberry juice drink (375, 716, 1131, 1396, 1741 mg of total flavan-3-ols) or an isocaloric control drink with one-week washout. Blood samples were taken at 0, 1, 2, 4, 6, 8 and 24 h; urine samples were collected at baseline, 0-8 and 8-24 h after drink intake. Plasma and urine were analyzed by UHPLC-ESI-QqQ-MS/MS. Metabolite identification and quantification were performed in comparison with calibration curves of in-house synthesized standards, when available.

RESULTS: Several phenyl- γ -valerolactones were identified in plasma and urine. Quantitatively, 5-(4-hydroxyphenyl)- γ -valerolactone-3-glucuronide, 5-(3-hydroxyphenyl)- γ -valerolactone-4-glucuronide and 5-(hydroxyphenyl)- γ -valerolactone-sulfate were the main metabolites. These compounds reached maximum plasma concentration (C_{max}) at about 4-6 h (T_{max}) and, consistently with this, the urinary excretion increased mainly during the 0-8 h collection period. Plasma and urinary concentrations increased in relation to the amounts of cranberry flavan-3-ols provided by the drink, showing a clear and linear dose-dependent effect. A high inter-individual variability in circulating and urinary metabolite levels was observed among subjects.

CONCLUSIONS: Glucuronide- and sulfate-conjugated forms of 5-(3,4-dihydroxyphenyl)- γ -valerolactone were relevant circulating metabolites after cranberry juice intake, exhibiting a dose-response relationship. These results strengthen the evidence that these compounds could serve as biomarkers of flavan-3-ol intake.

KEYWORDS: Cranberry; phenyl- γ -valerolactone; phenolic metabolite; bioavailability; metabolism; dose-response; inter-individual variability

A black and white photograph of raspberries, showing the texture of the fruit and the leaves. The raspberries are in the foreground, slightly out of focus, with more raspberries and leaves in the background.

Thursday, May 9th - Scientific Presentations

Gut Health & Gut Microflora



Dr. Jess Reed
University of Wisconsin-Madison

Session Chair
Current Research Review

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

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



Daniele Del Rio, PhD
University of Parma

Daniele is the Head of the University of Parma School of Advanced Studies on Food and Nutrition at the University of Parma, in Italy. He also acts as Scientific Director of the Need For Nutrition Education/Innovation Programme Global Centre for Nutrition & Health, in Cambridge, UK. He serves as Editor in Chief of the International Journal of Food Sciences and Nutrition (Taylor & Francis). He is a proud Commendatore (Knight Commander) of the Order of Merit of the Italian Republic, and he is proudly growing a team of brilliant scientists.

Phenyl- γ -valerolactones and Phenylvaleric Acids, the Main Colonic Metabolites of Flavan-3-ols: Their Relevance in Berry Health Benefits

Phenyl- γ -valerolactones (PVLs) and their related phenylvaleric acids (PVAs) are the main circulating metabolites of flavan-3-ols, the major class of flavonoids in the human diet and vastly present in berry fruits, cranberries in particular. Despite their presumed importance, these gut microbiota-derived compounds have, to date, been considered subordinate to their parent dietary compounds, flavan-3-ol monomers and proanthocyanidins. The role and prospects of PVLs and PVAs as key metabolites in the understanding of the health features of flavan-3-ols will be critically assessed and, among the covered topics, the formation, bioavailability and pharmacokinetics of PVLs and PVAs from different types of flavan-3-ols will be discussed, taking into account in vitro and animal studies, as well as inter-individual differences and the existence of putative flavan-3-ol metabolotypes. Being, as said, PVLs particularly represented in cranberry products, the most recent studies describing their bioavailability and contextual bioactivity after cranberry intake will be presented and discussed. Figure 1 shows a schematic representation of the main frames and outcomes considered for the assessment of PVL and PVA bioactivity.

Keywords: flavan-3-ols, phenyl- γ -valerolactones, cranberries.

References:

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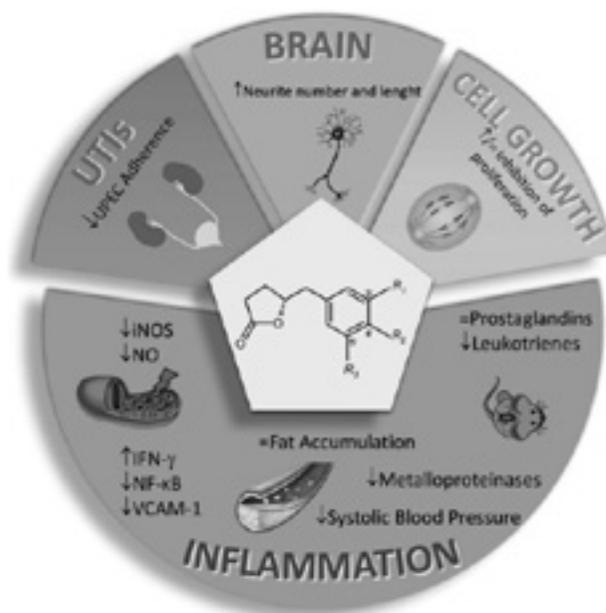


Figure 1



Dr. Franck Carbonero
University of Arkansas

Dr. Carbonero received his B.S. degree in Biology from Universite Joseph Fourier, France, his M.S. degree in Ecology from Universite Blaise Pascal, France and his Ph.D. degree in Microbiology from the University of Warwick, United Kingdom. He worked as a Postdoctoral Research Associate at the University of Illinois Urbana-Champaign for three years. He has served on the faculty (Assistant Professor) in the Department of Food Science at the University of Arkansas since 2013.

His research program is focused on nutrition and its impact on the human and animal gut microbiome, with focus on dietary bioactives. Dr. Carbonero has published 40 scientific articles and has delivered over 15 presentations at scientific meetings. He is an Academic Editor for Plos One and Editorial board member for 4 other journals.

Impact of Cranberry Juice Consumption on Gut and Vaginal Microbiota in Post-Menopausal Women

Authors: Ayoub Al Othaim¹, Daya Marasini¹, and Franck Carbonero¹

Affiliations:

1. Department of Food Science, University of Arkansas

Cranberries have long been purported to provide protection against Urinary Tract Infections (UTI). Recently, systematic reviews of human dietary intervention have been mixed with studies reporting positive effects. Polymeric cranberry polyphenol compounds are poorly bioavailable, and it is known that gut microbiota derived metabolites have higher bioavailability and efficiency. Causal pathogens related to UTI might be seeded from the gut or possibly vaginal reservoirs. We tested the hypothesis that cranberry intake would reshape bacteria taxa in the gut and possibly vaginal tract. To test the hypothesis, we enrolled 25 post-menopausal women (generally more at risk for UTI and with less fluctuation in gut and vaginal microbiota profiles due to ovarian cycles) into a double-blind, cross-over, randomized, placebo-controlled dietary intervention. Stool samples and vaginal swabs were collected at baseline and after 2 weeks of consumption of placebo or cranberry juice drinks, and microbiota analyses were performed by Illumina Miseq sequencing following a double index 16S rRNA gene amplicon strategy and the Mothur pipeline. Gut microbiota showed a high degree of individuality even if all samples generally fell in the Bacteroides enterotype as has been often reported for aging gut microbiota, and as a result no significant impact of the juices was detected on general profile or diversity. However, after removing one subject with significantly more diverse baseline profile, significant increases of *Prevotella*, *Clostridium XIVa* members, *Eggerthella* and *Bifidobacterium* were shown with the cranberry juice treatment; indicating positive modulation of the gut microbiota. Baseline vaginal microbiotas fell in three distinct profiles, presumably indicative of general vaginal ecosystem health. The healthy vaginal microbiota harbored overwhelming dominance of *Lactobacillus*; and both treatments resulted in marginal changes with slight increase of *Prevotella*. The second group was characterized by more diverse composition with several abundant taxa, with the diversity maintained by the cranberry juice but strongly decreased by the placebo. In this group, the placebo induced a strong increase in *Prevotella*. In contrast, the cranberry juice was found to increase various Actinobacteria and Firmicutes taxa in a subject specific manner. Finally, a third of the subjects were characterized by strong *Streptococcus* dysbiosis, which was partly resolved by the placebo and completely by the cranberry treatment. Again, the placebo increased the number of *Prevotella*, while the cranberry juice resulted in Actinobacteria and Firmicutes increases. Overall, these data suggests that cranberry consumption may improve vaginal microbiota composition in dysbiosed individuals, and that a combination of gut-borne *Prevotella* and *Bifidobacterium* (maybe other Actinobacteria and Firmicutes) stimulation by cranberries' carbohydrates and polyphenols respectively potentially mediate these beneficial properties; possibly by direct transfer from the gut to the vaginal ecosystem.

Keywords: Cranberries; Urinary Tract Infection, Gut microbiota, Vaginal microbiota; Dysbiosis

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Acknowledgement: Funding and cranberry products were provided by Ocean Spray Cranberries Inc.



Dr. Hang Xiao
University of Massachusetts, Amherst

Dr. Hang Xiao obtained his Ph.D. from University of Wisconsin-Madison, and had a post-doctoral training at the Rutgers University. Currently, Dr. Xiao is an Professor in the Department of Food Science at the University of Massachusetts. His long-term research goal is to develop food-based strategies for the prevention of major chronic diseases in humans such as colitis and colon cancer. Currently, Dr. Xiao is working on identifying potential disease preventive food components, and elucidating their molecular mechanisms.

Dr. Xiao has published more than 180 peer-reviewed manuscripts, and his research has been supported by funds from NIH, USDA, NASA, and NAS. Dr. Xiao has received multiple research awards such as Fellow of Agricultural and Food Chemistry Division of American Chemical Society (ACS), Mary Swartz Rose Young Investigator Award from American Society for Nutrition (ASN), and Samuel Cate Prescott Research Award from the Institute of Food Technologists (IFT), and International life Science Institute (ILSI) North America Future Leader Award. Dr. Xiao serves as the Associate Editor of Food & Function, Comprehensive Reviews in Food Science and Food Safety, and Journal of Food Science.

Promoting Colon Health by Berries: the Case Studies of Strawberry and Cranberry

There are growing interests in using whole food-based approach to prevent chronic diseases due to potential synergistic interactions among different bioactive components within the whole foods. Our recent research aimed to promote colon health by using whole berries to prevent colonic inflammation and colon tumorigenesis. In dextran sulfate sodium (DSS)-treated colitic mice, dietary whole strawberry (WS) reduced the disease activity index (DAI), prevented the colon shortening and spleen enlargement, and alleviated the colonic tissue damages. The abundance of pro-inflammatory immune cells was reduced by dietary WS in the colonic mucosa, which was accompanied by the suppression of overproduction of pro-inflammatory cytokines. Moreover, dietary WS partially reversed the alteration of gut microbiota in the colitic mice by increasing the abundance of potential beneficial bacteria and decreasing the abundance of potential harmful bacteria. In azoxymethane (AOM) and DSS-treated mice, dietary administration of whole cranberry (WC) significantly suppressed colon tumorigenesis as indicated by the reduced tumor incidence, multiplicity, burden and average tumor size in WC-fed mice compared to the positive control mice. Both gene and protein expression levels of pro-inflammatory cytokines IL-1, IL-6 and TNF- were markedly attenuated by WC treatment in the colon of AOM/DSS-treated mice. Moreover, WC profoundly modulated multiple signaling pathways/proteins related to inflammation, cell proliferation, apoptosis, angiogenesis and metastasis in the colon, which was closely associated with the inhibitory effects of WC on colon tumorigenesis. Overall, our results demonstrated chemopreventive effects of WS and WC against colonic inflammation and tumorigenesis in mice, respectively, providing a scientific basis for using whole berries as functional food to promote colon health in humans.



Dr. Yves Desjardins Laval University

Yves Desjardins is full professor at the Plant Science department and he is affiliated with the Institute of Nutrition and Functional Foods at Laval University, Québec Canada. He was recently appointed Dianafood-NSERC Industrial Chair on prebiotic effects of fruits and vegetables (PhenoBio). Trained in plant physiology, he is conducting research on phytochemistry and functionality of bioactive compounds from plants. He is PI or collaborator on many major preclinical and clinical studies on type-2 diabetes, cognitive decline, low-grade inflammation, urinary tract infection, skin diseases, and oral infections. Over the years, he has accompanied many horticultural and food processing companies in the development and the validation of the health benefits of horticultural commodities (e.g. Glucophenol, Neurophenol).

At the international scene, he is recognized for his innovative research program on health effects of fruits and vegetables. He was the Chair first International Symposium on Health Effects of Fruits and Vegetables in Québec City (FAVHEALTH 2005) and the OECD Symposium - "Emerging Topics in Health Effects Fruit and Vegetables" in Lisbon, Portugal (2010). He was nominated in 2005 as Chair of the ISHS Commission Fruit and Vegetable and Health a position he occupied until 2010 after being elected on the Board of ISHS. He organized in 2016 the International Strawberry Symposium (750 participants), which made a large place to health effects of this fruit. Recently (October 2017), he also organized the leading International Congress on Polyphenols and Health (www.ICPH2017-Québec.org) (>350 world renowned participants). His leadership in the field is recognized worldwide; he has been invited on numerous occasions to give keynote lectures at international meetings over the last few years (>20). He is involved in many international research projects in France, Mexico, Italy, and Belgium. Dr. Desjardins has written more than 120 publications (Normalized H-index of 23.5) and has trained many graduate students at the Master (29), at the Ph. D. (10) and the Postdoctoral level (12) since the beginning of his career. He sat on numerous master and Ph.D. thesis review (more than 67) and has participated as an examiner on more than 21 Ph.D. thesis evaluation committee in Canada and internationally. Since 2013, he filed 3 patents.

The Tannin Fractions of Polyphenol Berry Extracts Regulate Glycemia Through a Modulation of the Gut Microbiota and an Improvement of Gut Barriers Function

Authors: Yves Desjardins¹, Maria Carolina Rodriguez Daza¹, Fernando F. Anhe^{1,2}, Thibault V. Varin^{1,2}, Geneviève Pilon^{1,2}, André Marette^{1,2}, Stéphanie Dudonné¹, and Denis Roy¹

Affiliations:

1. Institute of Nutrition and Functional Foods, Laval University (Québec, Canada)
2. Department of Medicine, Faculty of Medicine, Cardiology Axis of the Québec Heart and Lung Institute, Laval University (Québec, Canada);

We have recently shown that berry polyphenols can strongly alter the gut microbiota and favour the development of a beneficial gut microbial community. In particular, they can stimulate the growth of a mucus inhabiting bacteria called *Akkermansia muciniphila*, coined the “anti-obesity” bacteria (Anhe et al., 2015). This bacterium has been associated with reduction of gut leakiness and attenuation of low-grade inflammation (de Vos, 2017). Some of our recent research results provide clues on how polyphenols can affect this bacteria and which fractions is responsible for this effect. Indeed, we have recently shown that polyphenol rich berries display remarkable capacity to modulate the microbiota and provide metabolic benefits. We analyzed the effects of Arctic and Nordic berries (cranberry, blueberry) in a model of diet-induced obesity. C57Bl/6J mice were fed a high fat/high sucrose diet and orally treated with berry extracts (200 mg/kg) for 8 weeks. Our results revealed that daily treatment with extracts of cloudberry, alpine bearberry and lingonberry, but not bog bilberry or crowberry, improved fasting insulin resistance and post-prandial hyperinsulinemia, findings associated with reduced hepatic triglyceride deposition and improved hepatic insulin clearance (Anhe et al., 2017). However, we could not completely reproduce these effects with blueberry and cranberry. The beneficial effects of these three extracts were associated with blunted intestinal inflammation and reduced lipopolysaccharide leakage. Similarly, blueberry and cranberry improve the thickness of the mucus layer and increase the number of goblet cells. The different polyphenols and in particular those containing a high concentration of procyanidins polymers were associated with major taxonomic alterations (e.g. increase in *Oscillibacter*, *Oscillospira*, *Ruminococcus*, *Christensenellenceae*, *Peptostreptococcaceae*, *Akkermansia muciniphila*, and *Adlercreutzia*) in the gut microbiota. We also strived to identify which fraction was responsible for the beneficial response of blueberry and cranberry PAC. We will show that the functional changes of microbial metabolic pathways induced by PAC are mostly implicated in xenobiotic metabolism. We speculate that tannins and polyphenols might interact with xenobiotic receptors (Aryl hydrocarbon receptors) and induce specific immune response, the production of Th17 regulatory cells producing anti-inflammatory cytokines (i.e., IL-22) leading to improved gut barrier integrity.

Keywords: Procyanidins, Microbiota *Akkermansia muciniphila*, Gut barrier function, Immune response

References:

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Thursday, May 9th - Scientific Presentations



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



Navindra Seeram, PhD
University of Rhode Island

Session Chair

Current Research Review

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

His research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants and their derived natural products for preventive and therapeutic effects against chronic human diseases. Dr. Seeram has co-authored over 165 original peer-reviewed research articles, 10 review-type articles, 17 book chapters, and 6 international patents. He has co-edited 3 books and is the founding editor of the Clinical Pharmacognosy book series published by CRC Press/Taylor and Francis. He serves on the advisory board of the American Botanical Council and on the editorial advisory boards of the Journal of Agricultural and Food Chemistry, the Journal of Berry Research, and the International Journal of Applied Research in Natural Products.

He was the recipient of the 2009 Young Scientist Award from the Division of Agricultural and Food Chemistry of the American Chemical Society and the 2017 Chair of that Division. He is among the most highly cited scientists in Agricultural Sciences by Thomson Reuters in 2014-2018 (based on Web of Science indexed citations from 2002-2018) 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.



Luke Howard, PhD
University of Arkansas

Dr. Howard received his B.S. degree in Horticulture from Purdue University, and his M.S. and Ph.D. degrees in Food Science from the University of Arkansas. He worked as an Analytical Chemist at the Dole Packaged Foods Research and Development Center for two years, and was an Assistant Professor in the Horticultural Sciences Department at Texas A&M University for five years. He has served on the faculty in the Department of Food Science at the University of Arkansas since 1997 (Associate Research Professor 1997-2002, Professor 2002-present).

His research program is focused on extraction and characterization of bioactive compounds in fresh and processed fruits and vegetables, with emphasis on berries. Dr. Howard has published over 120 scientific articles and five book chapters and has delivered over 90 presentations at scientific meetings. He is a Professional Member of the American Chemical Society.

Anti-proliferative Activity of Berry Volatiles

Authors: Luke Howard, Cindi Brownmiller, & Sun-Ok Lee

Affiliation: Department of Food Science, University of Arkansas, Fayetteville, AR

Berries are rich source of phytochemicals, especially polyphenolics [1] that are thought to protect against a number of chronic diseases including various types of cancer, cardiovascular diseases, diabetes and obesity, metabolic syndrome, and digestive disorders [2]. Berries also contain volatile compounds comprising a complex mixture of monoterpenes, ketones, acids, esters, furans, aldehydes, alcohols and lactones that are responsible for the unique aromas of berries [3,4]. However, information is lacking on volatile composition of berries and their potential health benefits.

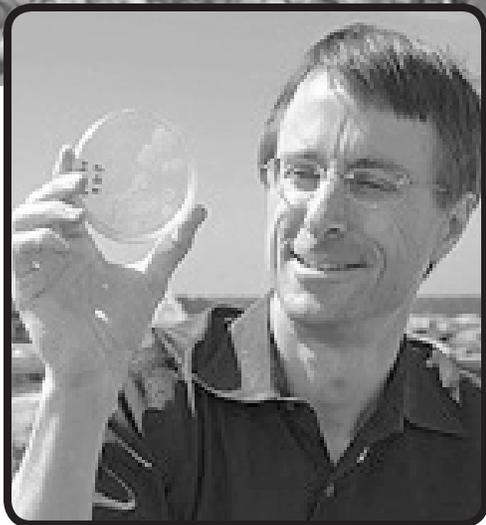
In this study we isolated phenolic and volatile fractions from fresh blueberries, blackberries, red raspberries, black raspberries, cranberries and strawberries. Black raspberries had the highest levels of total phenolics followed by cranberries, blueberries, blackberries, red raspberries, and strawberries. Blackberries had the highest levels of volatiles followed by black raspberries, strawberries, cranberries, red raspberries, and blueberries. Each berry contains a complex fingerprint of volatiles that impart unique aromas. Monoterpenes predominated in black and red raspberries, blueberries, cranberries, and strawberries, while acids predominated in blackberries. Phenolic and volatile fractions were evaluated for anti-proliferative activity using Caco-2 colon cancer cells at concentrations close to those found in fresh berries. The percentage inhibition of cell proliferation compared with the control for the phenolic fraction ranged from 3% for black raspberry to 58% for blueberry, whereas the values for the volatile fraction ranged from 5% for blackberry to 43% for black raspberry. The volatile fraction inhibited cellular proliferation much better than the phenolic fraction at 12 hr, whereas the phenolic fraction generally showed greater inhibition after 24 hr.

Berry volatiles showed comparable anti-proliferative activity as phenolics despite being present in the fruit at 300 to 2700-fold lower concentrations. In addition to impacting aroma of berries, volatile compounds may play an important role in health-promotion. More research is needed to determine the bioavailability and mechanisms responsible for anti-proliferative activity of berry volatiles.

Keywords: Anti-proliferation, Berries, Phenolics, Volatiles

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Dr. David Rowley
University of Rhode Island

Dr. David Rowley is a Professor of Biomedical and Pharmaceutical Sciences, Alex & Ani Positive Impact Laboratory, College of Pharmacy, University of Rhode Island

Dr. 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.

Dr. Rowley'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, Dr. Rowley is currently investigating molecular mechanisms by which cranberry constituents prevent urinary tract infections.



Sweet Health Prospects for Cranberry Sugars

The North American cranberry *Vaccinium macrocarpon* contains a cocktail of health friendly phytochemicals that provide potential benefits against inflammation, infection, and cardiovascular ailments. Most attention to date has focused on the impressive array of polyphenolic compounds found in cranberry, including the unique 'A-type' proanthocyanidins that are attributed with preventing bacterial attachment to epithelial cells. Until recently, the carbohydrate constituents of cranberry, especially short chained oligosaccharides, have received less scientific scrutiny. New evidence suggests that this cranberry component also provides a bevy of health benefits, including 'prebiotic' and anti-infective effects. Cranberry complex carbohydrates termed xyloglucans are likely not digested prior to reaching the colon and serve as nutrient sources to support healthy microbiomes. Bifidobacteria have been found to utilize these substrates and the products of this metabolism may impact human host health through energy and metabolite production, as well as support the growth of other beneficial bacterial strains. Cranberry xyloglucans have also been shown to reduce the production of microbial biofilms formed by uropathogenic *Escherichia coli* and interfere with attachment to human cells. New evidence shows that cranberry pectic oligosaccharides can prevent the 'quiescent' state of uropathogenic *E. coli*, suggesting a role in the prevention of recurrent urinary tract infections. Other in vitro studies have shown that cranberry complex carbohydrates possess free radical scavenging effects and prevent the formation of advanced glycation endpoints, suggesting a possible role in limiting oxidative stress and inflammation. Taken together, mounting scientific evidence supports that complex carbohydrates from cranberry should not be overlooked when considering the health benefits of this North American fruit.

Keywords: Cranberry, Oligosaccharide, Urinary Tract Infection, *E. coli*, quiescence



Li-Shu Wang, PhD
Medical College of Wisconsin

Dr. Wang is an Associate Professor of Medicine, Medical College of Wisconsin. She received her Ph.D. in Veterinary Biosciences in the College of Veterinary Medicine at Ohio State University in June, 2006. Currently, she is an editorial member for *Journal of Berry Research* and *e-Food Journal*. Dr. Wang has published in high-rank journals in the field of cancer prevention such as *Clinical Cancer Research*, *Carcinogenesis*, *Cancer Prevention Research*, *Cancer Immunology Research*, *Frontiers in Immunology*, *International Journal of Cancer*, etc. Dr. Wang's expertise is in colorectal cancer (CRC) biology and prevention in animals and humans.

The major goal of Dr. Wang's research is to translate the findings from bench to bedside. Using bio-directed fractionation, Dr. Wang showed that the anthocyanins in black raspberries (BRBs) are important for their chemopreventive effects and she provided evidence that the ellagitannins may be less important. More importantly, she has evidence that BRBs cause demethylation of tumor suppressor genes in rodent and human colon leading to their enhanced expression in two human clinical trials. The protective effects of BRBs against human and mouse CRC are associated, at least in part, with their hypomethylation activities. Loss of responses to berry treatment in humans may be due to decreased sensitivity to berry-induced DNA demethylation. Further, BRB intervention induces significant metabolic changes and affects energy generating pathways in CRC patients.

Recently, Dr. Wang's laboratory is investigating the mechanisms of active metabolites from BRBs to influence colon and pancreatic cancer immunology through epigenetic modifications. The results from animal models of both cancer types indicate that berries dampen tumor-induced immune suppressive microenvironment by decreasing CD11b+ myeloid cells, and boosting CD8+ T-cell and natural killer cells. In an effort to translate laboratory findings to clinics, Dr. Wang's group is investigating the effects of BRBs on DNA methylation in patients with myelodysplastic syndrome (MDS). The aim of this pilot clinical trial is to evaluate the hypomethylating properties of BRBs in MDS patients after 3 months of BRB supplementation.

Are We Ready for Precision Cancer Prevention Using Black Raspberries?

Authors: Pan Pan, Hayden Krause, Arielle Baim, Lyndsey Runaas, Laura Michaelis, Ehab Atallah, Li-Shu Wang

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The importance of diet and nutrition in the etiology of some cancers is appreciated. However, the exact nature of how diets impact cancers is not clearly understood. More importantly, significant inter-individual variability can make the outcome of dietary interventions less predictable. We have encountered non-responders in our clinical trials using black raspberries (BRBs) and we are investigating the mechanisms for the non-responsiveness to BRB-intervention.

Familial adenomatous polyposis (FAP) is characterized by the early onset of colonic polyposis and a high risk for colorectal cancer [1]. FAP is treated by colectomy followed by lifelong removal of rectal polyps. Nonsteroidal anti-inflammatory drugs (NSAIDs) were first reported to cause regression of colonic polyps in subjects with FAP over two decades ago. Case series and many randomized controlled trials have confirmed this observation for nonselective NSAIDs such as sulindac [2-5]. However, the increased risk of cardiovascular, thromboembolic, and cerebrovascular events led to the withdrawal of rofecoxib from the market and remains a concern for celecoxib [6]. Our group conducted a human clinical study to determine whether BRBs might regress rectal polyps in patients with FAP [7]. Fourteen patients with FAP were treated with BRBs daily for 9 months. Despite the encouraging finding that a 9-month BRB intervention decreased polyp burden in 11 of 14 FAP patients, 3 patients were non-responders. Mechanistic studies suggest that non-responders were resistant to BRB-induced hypomethylation.

Myelodysplastic syndromes (MDS) are a group of bone marrow disorders characterized by progressive cytopenias and progression to acute myeloid leukemia [8]. Hypomethylating agents (HMAs), such as azacitidine and decitabine, are FDA-approved therapies for MDS patients [9-10]. Approximately 50% of MDS patients respond to HMAs. Although both azacitidine and decitabine are effective therapies for some MDS patients, they are associated with significant toxicities that place a large burden on MDS patients and affect their quality of life. We conducted a pilot clinical trial to evaluate the hypomethylating properties of BRBs in patients with low-risk MDS. Study is still ongoing and we have recruited 18 MDS patients. Data from 7 patients suggested that 3 months of BRB intervention induced hypomethylation in 6 out of 7 patients, 1 patient was a non-responder.

Therefore, data from our two clinical trials suggest that changes of methylation maybe used as indicators for responsiveness to BRB intervention that warrants investigation in larger clinical trials. It would be ideal to identify individuals who are likely to benefit from a BRB intervention before recruiting subjects onto clinical trials.

Keywords: precision nutrition, cancer prevention, black raspberries, methylation

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Poster Presentation Abstracts



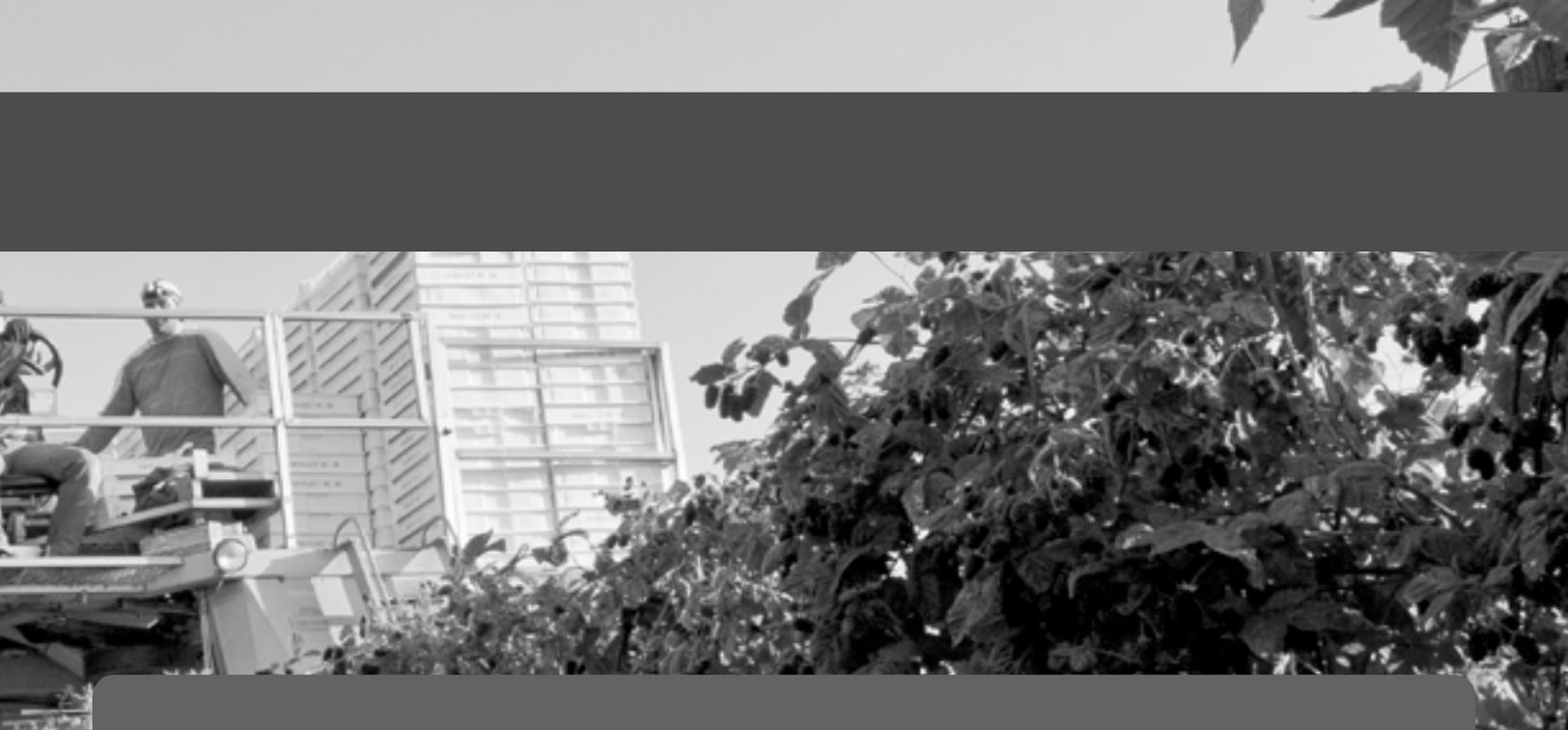
Poster Session Chair

Luke R. Howard, PhD
University of Arkansas

The **2019 Berry Health Benefits Symposium** is pleased to offer two ways for students of berry science to share their latest research findings. Graduate students and postdoctoral fellows were invited to submit abstracts for review and consideration for both Poster Presentations and Oral Presentations at this years symposium.

These **Junior Investigators** submitted original research relating to the symposium themes of berry biochemical composition, cardiovascular health, metabolism regulation, brain aging, and other health properties of berry fruit.

The following posters were selected for Poster Presentations and are viewable in the **Gevirtz Room**. The abstracts for the 3 oral presentations are available on pages 46-48. The winner of the poster session will be announced at the Ocean Spray Keynote Dinner on Wednesday, May 7th.



Impact of Blueberry Polyphenols on Metabolic Disorders in Diet Induced Obese Mice

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BACKGROUND: Given the obesity epidemic, it is essential to identify strategies that could contribute to prevent this situation. Blueberry consumption can prevent obesity-linked metabolic diseases and it has been proposed that its polyphenol content may contribute to these effects. Indeed, polyphenols have been shown to favourably impact gut microbial populations and metabolic health, but the role of specific polyphenol classes remains elusive.

OBJECTIVE: We aimed to evaluate the impact of whole blueberry or blueberry polyphenols against the development of obesity and insulin resistance, and to demonstrate their prebiotic-like action by using fecal microbiota transplantation (FMT).

METHODS: In a first study, 70 C57BL/6 male mice were assigned to one of the following diets for 12 weeks: balanced diet (Chow), high fat high sucrose (HFHS) diet, or HFHS supplemented with either whole blueberry powder (BB), anthocyanidin (ANT) or proanthocyanidin (PAC)-rich extracts. After 8 weeks, mice were housed 24h in metabolic cages and an oral glucose tolerance test (oGTT) was performed at the end of the protocol. In a second study, 60 germ-free mice fed HFHS diet received FMT from one of the five groups above, twice a week, for 8 weeks followed by an oGTT.

RESULTS: In the first study, PAC-treated mice were leaner than HFHS controls, although they had the same energy intake. Interestingly, PAC-treated mice were more physically active, and this observation was reproduced in germ-free mice receiving FMT from these mice. PAC and ANT-treated mice had improved insulin sensitivity during oGTT, and this feature was also reproduced in germ-free mice following FMT. Moreover, germ-free mice receiving BB and ANT microbiota were leaner than HFHS controls.

CONCLUSION: These results show that blueberry polyphenols can reduce diet-induced body weight and improve insulin sensitivity, and that at least part of these beneficial effects are explained by the modulation of the gut microbiota.

KEYWORDS: Blueberry polyphenols, obesity, insulin resistance, fecal microbiota transplantation

Inhibitory Effects of Cranberry Polyphenol and Volatile Extracts on Nitric Oxide Production in LPS Activated RAW 264.7 Macrophages

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BACKGROUND: Cranberry polyphenols possess antioxidant and anti-inflammatory properties and are thought to protect against many chronic diseases. Cranberries also contain an array of volatile compounds responsible for the aroma of the fruit, which may also contribute to the berries anti-inflammatory effect.

OBJECTIVE: The objective of this study was to compare the inhibitory effects of polyphenol and volatile extracts isolated from cranberries and purified volatiles on nitric oxide (NO) production in lipopolysaccharide (LPS) activated RAW 264.7 macrophages.

METHODS: The inhibition of NO production of the fresh cranberry polyphenol and volatile extracts at 2, 4, and 8-fold dilutions of their original concentrations in fresh cranberries was evaluated before (1 hr) and after (24 hr) application of LPS using RAW 264.7 mouse macrophage cells. We also measured NO levels produced in vitro by RAW 264.7 cells when treated with the major terpenes -terpineol, linalool, linalool oxide, and eucalyptol before and after inducing inflammation using LPS. Polyphenols were analyzed by HPLC/HPLC MS and volatiles were analyzed by GC/GC-MS.

RESULTS: After inducing inflammation with LPS, the polyphenol treatments (317.8 and 635.7 µg/g) and 1.8 µg/g volatile treatment lowered NO levels 50-62% compared to the positive control (P<0.05). When the cells were treated with polyphenol and volatile extracts before inducing inflammation, the 635.7 µg/g and 317.8 µg/g polyphenol treatments and 1.8 µg/g and 0.9 µg/g volatile treatments lowered NO levels (13-52%) compared to the positive control (P<0.05). -terpineol at a concentration found in fresh cranberries (1.16 µg/g) was demonstrated to act both as an effective preventer and treatment for pre-existing inflammation.

CONCLUSIONS: Polyphenol and volatile extracts from cranberry acted both as an effective preventer and treatment for pre-existing inflammation. Future studies are needed to reveal the mechanisms by which volatile compounds, especially -terpineol act to prevent and reduce inflammation and to determine the bioavailability of terpenes.

KEYWORDS: Alpha terpineol, Anti-inflammatory, Cranberry, Polyphenols, Volatiles

The Effects of Acute Wild Blueberry (WBB) on Cognition, Mood and Event-Related Potentials (ERPs) in Healthy 7-10 Year Old Children

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BACKGROUND: Previously, short-term improvements in executive functioning (EF), verbal memory and mood have been observed 1.5, 2, 3 and 6 h following consumption of blueberry (BB) based beverages (127-253mg anthocyanins) in typically developing 7-10 year olds (Khalid et al, 2017; Whyte and Williams, 2015; Whyte et al, 2016; 2017). However, the mechanism of action underlying such acute effects remains to be elucidated. Flavonoids are thought to cross the blood-brain-barrier and increase cerebral blood flow, and may exert direct effects on the brain regions involved in cognitive processes.

OBJECTIVE: The aim was to investigate whether cognitive benefits following BB consumption are underpinned by changes in regional electrophysiological activation as assessed with event related potentials (ERP) from electroencephalography (EEG).

METHODS: EF, inhibition and mood were assessed in 14 healthy children, alongside ERP recordings, 2 h following consumption of either a freeze-dried wild BB drink (253mg anthocyanins/766mg polyphenols) or a sugar and vitamin C matched control (13mg polyphenols) in a crossover design (1 week washout).

RESULTS: Behavioural benefits were observed on EF measures for children who consumed BB at the first visit, in comparison to those consuming placebo first. Specifically, improved accuracy and reaction time for cognitively demanding trials was seen. Furthermore, these benefits coincided with higher electrophysiological activation in frontal brain areas associated with inhibition.

CONCLUSIONS: Overall, this data set suggests wild BB could be an effective short-term intervention for children, to improve initial learning in a situation requiring EF abilities. This study is one of the first to converge behavioural and electrophysiological data after consumption of BB flavonoids, and provides further understanding of potential acute mechanisms of action.

KEYWORDS: Blueberry, anthocyanins, cognition, inhibition, executive function, children, neuroimaging

Strawberry Enriched-Diet Counteracts Oxidative Stress and Inflammation in Rats Stressed with Lipopolysaccharide or Doxorubicin

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BACKGROUND: An excessive production of free radicals leads to oxidative stress and inflammation, that represent a common denominator in the pathogenesis of most chronic diseases. In the last years, many epidemiological studies showed that ROS production can be efficiently counteracted through a diet rich in fruits and vegetables. Strawberry, thanks to high content in vitamin C, folate and phytochemicals, represents one of the most important source of bioactive compounds which exert essential biological properties against oxidative stress, inflammation, cancer, cardiovascular and neurodegenerative diseases.

OBJECTIVE: The aim of the present study is to evaluate the protective effects of strawberry consumption against oxidative stress and inflammation induced by doxorubicin (Dox) or lipopolysaccharide (LPS) injection, respectively.

METHODS: Young Wistar male rats were fed with strawberry enriched diet (15% of the total calories) for two months and Dox (10 mg/kg; i.p) or LPS (10 mg/kg; i.p.) were injected at the end of the experimental period. ROS levels, oxidative damage biomarkers, inflammatory biomarkers, antioxidant enzyme activities were assessed in plasma and liver, together with liver mitochondrial antioxidant levels and functionality.

RESULTS: In rats stressed with Dox or with LPS, strawberry consumption significantly reduced ROS and nitric oxide production as well as lipid (hydroperoxides, TBARS) and protein (carbonyls) oxidation and reestablished antioxidant enzymes activities (SOD, catalase, glutathione reductase, transferase and peroxidase) and mitochondrial functionality (respiration and glycolysis rate) by modulating AMPK/Nrf2 pathway, restoring values similar to those of control group. At the same time, a reduction in gene expression of some inflammatory-cytokines, as IL-1, IL-6 and TNF- and in their related pathways as NF-kB and iNOS has been found.

CONCLUSION: The results obtained confirm the in vivo potential health benefit of strawberry fruit against oxidative and inflammatory disorders, thanks to the high content of bioactive compounds of this fruit.

KEYWORDS: oxidative stress, inflammation, strawberry, bioactive compounds, healthy diet.

Berry Consumption in Older Adults: Altered Gut-Microbiome Composition and Diversity

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BACKGROUND: Evidence is emerging regarding the modulatory activity of dietary polyphenols on the gut microbiome, but the influence is still poorly understood. In turn, the gut microbiota is known to contribute to the production of polyphenol metabolites. Moreover, recent evidence suggests that gut microbiome diversity relates to many health outcomes, such as cognitive function.

OBJECTIVE: We quantified associations between berry consumption and gut microbial diversity and composition. We hypothesized that a positive linear relation exists between berry consumption and gut microbial diversity. Based on existing literature, we further hypothesized that berry consumption would be associated with Enterobacteriaceae and Clostridium, specifically.

METHODS: In the parent study, we conducted 3-day diet recalls and cognitive assessments with 83 healthy adults aged 66-82y, and participants provided fecal samples. Total servings of berries were calculated using the Nutrition Data System for Research (Minneapolis, MN). DNA was isolated from fecal samples, and 16S rRNA sequence were processed to assign taxonomy and to calculate the Shannon Diversity Index, a measure of within-person microbial diversity.

RESULTS: Among participants who consumed berries (n=22; servings=0.13-4.87), consumption was significantly positively related to the Shannon Diversity Index, [F(2,18)=4.310, p=.03, r²=.324]. Further, berry consumption was associated with higher relative abundance of Enterobacteriaceae and Clostridium spp. (p=.026 & p=.046, respectively).

CONCLUSIONS: In this analysis of a free-living human sample, berry consumption was positively associated with gut microbial diversity. Further, we confirmed associations between specific genera and polyphenols in controlled studies previously reported by others. The inclusion of berries in the daily diet may contribute to increases in the diversity of gut microflora, which in turn may lead to improvements in related health issues such as inflammatory disease and cognitive dysfunction. Future studies and analyses will further refine our understanding of the interplay among berries, influential gut microflora, and health outcomes.

KEYWORDS: Berries, Gut, Health, Microbiome, Microflora, Polyphenols

Leveraging a High-Throughput Phenotyping Method to Study Anthocyanin Genetics in Blueberry (*Vaccinium* sp.)

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BACKGROUND: Blueberry is a well-recognized health protective fruit with functionality derived from micronutrient and phytochemical density, particularly anthocyanins. Despite their importance, little is known about the genetic mechanisms controlling anthocyanin accumulation in this crop.

OBJECTIVE: The aims of this study were to (1) develop an ad-hoc phenotyping method for screening the total anthocyanin content (TAC) in blueberry, and (2) evaluate the variation in TAC within a genetic mapping population.

METHODS: A total of 57 genetically diverse blueberry genotypes were processed and scanned using a Perten DA 7250 near-infrared (NIR) analyzer. The resulting spectra were combined with corresponding reference analysis data (high-performance liquid chromatography) to produce a calibration, which was further optimized to reduce error using a partial least squares approach. The calibration was then used to predict TAC (ug/mg dry weight (DW) skin tissue) within a biparental mapping population (cv. Reveille x cv. Arlen) harvested in 2017 (n=169) and 2018 (n=194). Variance components (genotype, year, genotype by year) were calculated using a linear mixed model analysis, which were used to determine broad-sense heritability for the anthocyanin trait in this population.

RESULTS: A comparison of observed and predicted values in the calibration dataset revealed the model accounted for 81% of observed variation in TAC ($R^2 = 0.8073$). Overall, TAC in the mapping population was greater in 2018 compared to 2017, with values ranging from 4.8 to 93.8 ug/mg DW in 2017 and 49.9 to 129.3 ug/mg DW in 2018. Using the calculated variance components, broad-sense heritability was estimated to be 41% for the anthocyanin trait.

CONCLUSIONS: It is feasible to use NIR for screening TAC in large numbers of blueberry genotypes. The observed variation in TAC of the mapping population provides support for the viability of conducting a genetic association mapping study.

KEYWORDS: NIR, anthocyanins, biparental mapping population, blueberry, *Vaccinium*, high-throughput, genetic studies

Influence of Genotype and Maturity on Phenolic Composition of Strawberry (*Fragaria X Ananassa* Duch.) Fruits

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BACKGROUND: Strawberries are rich in phenolic compounds, contributing both to sensory quality and health promoting properties of the berries. The composition and concentration of phenolic compounds will, however, vary with several factors such as genotype and maturity.

OBJECTIVE: The objective of the study was to determine the effects of genotype and maturity on phenolic composition of 10 strawberry genotypes in 4 maturity degrees; white-greenish, white with touch of red, turning red and fully red.

METHODS: Three biological replicates of each sample were freeze-dried, milled and extracted with 70% acetone. Phenolic compounds were characterized and quantified by HPLC-DAD-MSn as previously described (Aaby et. al, Food Chem. 2012, 132, 86-97).

RESULTS: The concentrations of anthocyanins and coumaroyl hexoses increased during maturation and the variation between the samples were mainly determined by maturity, explaining 79 and 65% of the variance, respectively. Total flavonol concentration was not affected by maturity, while agrimoniin and ellagic acid glycosides decreased during maturation, but were mainly affected by genotype, i.e. with 63 and 52% explained variance, respectively. In ripe berries, anthocyanin concentration varied from 12 - 44 mg/100 g. Pelargonidin-3-glucoside was the major anthocyanin in all genotypes (71-90%), while the occurrence of other anthocyanins varied more between the genotypes.

CONCLUSIONS: The concentrations of anthocyanins and coumaroyl hexoses varied mainly with maturity, while concentration of ellagic acid containing compounds were predominantly influenced by genotype.

KEYWORD: strawberry, maturity, genotype, polyphenols, anthocyanins, ellagitannin, flavonols, cinnamic acid

Comparison of Blueberry Extract vs. Blueberry Powder Confections: Exposure Analyzed by Targeted and Untargeted Metabolomics

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BACKGROUND: Blueberries are a rich source of bioactive flavonoids, mainly anthocyanins, that reach systemic circulation and can cross the blood-brain barrier. However, consumers would have to eat more than a cup of blueberries in order to consume an efficacious dose according to animal studies. It is unclear if anthocyanins from a phenolic extract of blueberries have similar bioavailability as from freeze-dried blueberries. Thus, our lab developed blueberry confections from concentrated blueberry extract (BE) and whole blueberry powder (BP) for use in a human dietary intervention.

OBJECTIVES: The objectives of this study were to compare the bioavailability of a single dose of anthocyanins from confections made with either BE or BP in the urine of 12 subjects using a cross-over design.

METHODS: A dietary intervention with blueberry confections was conducted for urinary uptake of anthocyanins and their metabolites in 12 human subjects. Confections were formulated with BP or BE (125mg anthocyanins/dose), sugar, jello, gelatin, citric acid, and water. Subjects followed a week wash-out period eliminating berries from their diet and a 24hr urine collection immediately followed by a single dose of one type of confection (6 pieces of BE or 4 pieces of BP confection) and another 24hr urine collection. A second washout was conducted with intervention using the alternate confection. Urine samples were tested in a targeted manner by mass spectrometry to determine anthocyanin metabolites and by NMR metabolomics with confirmation by mass spectrometry.

RESULTS/CONCLUSIONS: Anthocyanins were not detected in the 12 subject urines after the week-long washout. Following either intervention, all anthocyanins were found in all urines as well as glucuronide conjugates. Only 0.05% by weight of anthocyanins consumed were recovered in the urine samples and uptake varied among individuals more so than by confection type. NMR metabolomics revealed adipic acid, a component of commercial gelatin, as highly elevated in the confection groups.

KEYWORDS: Blueberry confections, Extract, Powder, Anthocyanins, Bioavailability, Metabolomics

Evaluation of Cranberry Proanthocyanidins in an In Vitro Model of Gut Barrier Dysfunction

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BACKGROUND: Cranberries (*Vaccinium macrocarpon*) have a beneficial effect on several aspects of human health. The present study investigated the effects of cranberry proanthocyanidins (c-PAC) on an in vitro model of gut barrier dysfunction (GBD) that monitors epithelial cell membrane integrity and macrophage activation during an *E. coli* challenge.

OBJECTIVES: The primary objective of this study was to understand if c-PAC can attenuate *E. coli* induced gut barrier dysfunction and subsequent macrophage activation and determine a range of effective concentrations.

METHODS: c-PAC were isolated from frozen cranberries using solid phase chromatography. An in vitro co-culture model that uses a pathogenic strain of *E. coli* to cause membrane disruption and subsequent macrophage activation was used to evaluate the ability of c-PAC to attenuate these responses. Tight junction integrity of the epithelial layer was assessed by measuring the transepithelial electrical resistance (TEER), while macrophage activation was evaluated by expression of inflammatory proteins as determined by western blot. TEER values were monitored every 24 hours after the *E. coli* challenge for a total of five days.

RESULTS: Our data indicates that when epithelial cells are challenged with *E. coli* in the presence of PAC from cranberries, the decrease in TEER was partially attenuated and macrophage expression of inflammatory proteins, cyclooxygenase-2 (COX-2) and tumor necrosis factor- (TNF-), were reduced to levels of the control. In addition, the restoration of membrane integrity is accelerated with the presence of c-PAC.

CONCLUSIONS: Cranberry PAC attenuate *E. coli* induced reduction in TEER and subsequent increase in COX-2 and TNF- protein expression in a dose dependent manner. These results help establish effective therapeutic doses and formulations for treatment of GBD that can be tested in translational animal models and early phase clinical studies.

KEYWORDS: cranberry, proanthocyanidins, transepithelial electrical resistance (TEER), macrophage activation, gut barrier dysfunction (GBD)

A Randomized, Double-Blinded, Dose-Response Study of the Acute Effects of Wild Blueberry Extract on Cognition, Mood and Blood Pressure in Older Adults

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BACKGROUND: Previous research has shown the benefits of flavonoid intake on cognitive performance in healthy populations across all age groups. In particular, supplementation with a flavonoid-rich wild blueberry extract improved episodic memory performance in older adults after three months.

OBJECTIVE: The primary research outcome of this study is to investigate whether the acute administration of a range of doses of wild blueberry extract are effective at maintaining episodic memory in healthy older adults. The secondary research outcomes of this study are to investigate the acute effects of a range of doses of wild blueberry extract on executive function, mood and blood pressure in the same population. It is hypothesised that wild blueberry extract will have dose-dependent beneficial effects on cognition, mood and blood pressure.

METHODS: A double-blind, counterbalanced, crossover design was used to compare the acute effects of four wild blueberry extract doses, containing 7 mg, 14 mg, 38 mg and 76 mg anthocyanins, with a sugar-matched placebo. Twenty-eight healthy older adults, aged 68-75 years completed the study. Measures of cognition, mood, and blood pressure were recorded at baseline, 2 hours, 4 hours and 6 hours post-intervention.

RESULTS: In an executive function task, the 7 mg dose significantly improved reaction times compared with placebo. Treatment with wild blueberry extract alleviated the significant decline in combined executive function scores at 4 hours observed with placebo. Compared to placebo, the 14 mg dose elicited significantly lower diastolic and systolic blood pressure. However, results for episodic memory and mood were more equivocal.

CONCLUSIONS: The findings provide evidence for improvement in executive function and blood pressure following acute supplementation with wild blueberry extract. The cognitive outcomes differ from previous literature, which suggests that episodic effects are most commonly observed in older adults following flavonoid supplementation. The blood pressure findings concur with the existing literature.

KEYWORDS: wild blueberry; flavonoid, anthocyanin; cognition; blood pressure; executive function; older adults

Angiogenesis is Differentially Modulated by Anthocyanin and Phenolic Extracts from Wild Blueberry (*V. Angustifolium*) through Pi3k Pathway

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BACKGROUND: Angiogenesis is a normal biological process that occurs in tissue development and is highly linked to wound healing and a plethora of pathological conditions such as atherosclerosis and diabetes.

OBJECTIVE: This study investigates the effect of anthocyanin (ACN) and phenolic acid (PA)-rich fractions and their combination (ACNs:PAs) from wild blueberry powder (*Vaccinium angustifolium*) on angiogenesis.

METHODS: Human umbilical vein endothelial cells (HUV-EC-C [HUVEC] (ATCC® CRL-1730™)) were used. ACN and PAs fractions were extracted from freeze dried wild blueberry powder and characterized by liquid chromatography. Angiogenesis assay was performed using the IBIDI μ -slide Angiogenesis plate. Gene expression was performed for VEGFA, NOS3, AKT1 and GAPDH. Western blot analysis was performed for AKT1, VEGF, eNOS and α -tubulin.

RESULTS: HUVECs were exposed to ACN and PA-rich fractions and their combination at 0.002 μ g/ml, 8 μ g/ml, 15 μ g/ml, 60 μ g/ml and 120 μ g/ml for a maximum of four hours. Cytotoxicity assays documented that ACNs at 1000 μ g/ml was toxic to HUVECs. The total number of meshes, area of meshes, number of nodes, number of master junctions and length of master segments, markers of angiogenesis were measured and analyzed using Image J with the Angiogenesis Analyzer plugin. ACNs appear to inhibit HUVEC tube formation (meshes) at 60 μ g/ml while PAs enhance endothelial tube formation at 0.002 μ g/ml, 60 μ g/ml and 120 μ g/ml. Lastly ACNs:PAs promote this process at 60 μ g/ml:60 μ g/ml for all parameters measured. ACNs reduced gene expression for eNOS and AKT. Gene expression was increased compared to the control with PAs for VEGF and AKT. ACNs:PAs increased VEGF and decreased eNOS and AKT. Western blot analysis documented increase levels of AKT only for PAs and ACNs:PAs after two hours of treatment.

CONCLUSIONS: Findings suggest that angiogenesis is differentially modulated based on the bioactive fraction and is concentration-dependent. Further investigation is necessary to determine the mechanism(s) behind this biological phenomenon with possible implications to atherosclerosis.

KEYWORDS: wild blueberry, anthocyanin extract, phenolic acid extract, angiogenesis, HUVECS, AKT1, VEGF, eNOS.

Dietary Intake of Berries and Risk of Cardiovascular Disease: A Meta-Analysis of Prospective Cohort Studies

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BACKGROUND: There is accumulating evidence that increased intake of polyphenols, such as anthocyanins, reduce risk factors associated with non-communicable and neurodegenerative diseases and berries can account for a considerable dietary contribution of these compounds.

OBJECTIVE: To systematically determine the association between berry intake and risk of cardiovascular diseases (CVD).

METHODS: A systematic literature search of prospective studies reporting anthocyanin intake and risk of fatal or nonfatal CVD was performed using SCOPUS, MEDLINE, CINAHL and Cochrane Library databases. Based on this search, the multivariate-adjusted relative risk (RR) or hazard ratio (HR) of the highest category of berry intake were pooled in a random-effects meta-analysis.

RESULTS: Seven studies involving 189,971 participants with more than 11,982 incidences of fatal or non-fatal CVD were included in the current meta-analysis. All studies used dietary history or food frequency questionnaires to determine berry consumption. The results of the meta-analysis suggested that a higher intake of berries was not associated with a reduced risk of; coronary heart disease (RR: 0.95; 95% CI: 0.75, 1.21; n = 2), total CVD (RR: 1.12; 95% CI: 0.80, 1.56; n = 2), or CVD mortality (RR: 0.95; 95% CI: 0.79, 1.13; n = 2) with little between study heterogeneity ($I^2 \leq 34\%$). Dietary intake of berries was also not associated with reduced risk of stroke (1.01; 95% CI: 0.85, 1.20; n = 5), but there was moderate heterogeneity between studies ($I^2 = 58\%$).

CONCLUSIONS: Despite experimental data suggesting berries reduce risk factors of CVD, epidemiological evidence does not support these associations, although berry intake has been linked to a number of other health benefits. It should also be acknowledged that only a limited number of cohorts with memory based dietary assessment methods were identified in the current meta-analysis, therefore further studies with better estimates of berry intakes are needed.

KEYWORDS: Berries, Anthocyanins, Cardiovascular disease, Epidemiology

Strawberry Methanolic Extract Inhibits Adipogenesis in 3T3-L1 Mouse Embryo Fibroblasts Cells

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BACKGROUND: Obesity is characterized by an expansion of adipose tissue mass resulting from increased adipocytes number (hyperplasia) and/or size (hypertrophy). The excessive accumulation of differentiated adipocytes is associated with high triglyceride levels and a general impairment of catabolic pathways. Scientific evidences have demonstrated that strawberry bioactive compounds are able to prevent the development of dyslipidemia and obesity, lipid peroxidation and LDL-cholesterol in different animal models.

OBJECTIVE: The main objective of the present work is to evaluate the effect of a strawberry extract (STWE) on 3T3-L1 pre-adipocytes differentiation.

METHODS: Differentiation of 3T3-L1 mouse embryo fibroblasts cells was induced 2 days after 100% confluence (Day 0) by adding 0.5 mM methylisobutylxanthine, 1 μ M dexamethasone, and 1 μ g/mL insulin. Two days later, the differentiation medium was replaced with DMEM supplemented with 10% FBS and 1 μ g/mL insulin, which was changed every 48 h for another 8 days. STWE was added at Day 0 and maintained during cell differentiation until Day 10 when the cells were harvested. Total cholesterol, LDL-cholesterol and triglyceride levels were determined by enzymatic colorimetric kits, while total lipid accumulation was assessed by Oil Red O staining on days 2, 6 and 10 after the induction. The expression of some genes involved in lipid metabolism has been also evaluated.

RESULTS: STWE significantly reduced 3T3-L1 pre-adipocytes differentiation, lipid accumulation and down-regulated the expression of the fatty acid binding protein (FABP4), the acetyl-CoA carboxylase (ACC) and the sterol regulatory element-binding protein (SREBP1). Likewise, it stimulated AMP-activated protein kinase (AMPK), sirtuin 1(Sirt1) and the liver kinase B1 (LKB1) in a dose-dependent manner.

CONCLUSION: Our results provide new insights into the molecular mechanism of the hypocholesterolemic effects of strawberry, supporting its further employment as functional foods and a potential hypolipidemic agent.

KEYWORDS: obesity, adipocytes, adipogenesis, strawberry, bioactive compounds, healthy diet

Natural Wax of Arctic Berries

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BACKGROUND: A hydrophobic layer called cuticle cover the aerial parts of land plants. The cuticle limits non-stomatal water loss and gives protection against external biotic and abiotic stresses. The layer is composed of the polymer cutin and a complex mixture of embedded waxes. In addition, bioactive secondary metabolites such as triterpenoids and phenolic compounds can be present. Due to unique properties, these natural waxes have a huge potential in food, cosmetics and pharmaceutical products. In Scandinavia, wild berries such as bilberry (*Vaccinium myrtillus* L.) are interesting candidates for domestic wax production. As for other aspects of plant growth the quantity and quality of the wax layer can be affected by different environmental factors such as relative humidity, soil moisture, sunlight, irradiance and temperatures. These are all factors that vary greatly in Scandinavia.

OBJECTIVE: How is the composition and amount of wax affected by the growth location?

METHODS: Berries were picked at three different locations in Norway, Finland and Latvia and wax were extracted and composition were analyzed by GC-MS.

RESULTS: The results are currently under analysis and will be presented at the conference.

CONCLUSIONS: The results from the study could reveal which areas that are best suited for future utilization of waste streams from wild berry production.

KEYWORDS: Wax, bilberry, *Vaccinium myrtillus*, circular economy, triterpenoids secondary metabolites

Wildberries – Prediction of Yield and Quality of Lingonberries

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BACKGROUND: There are increasing demands for wild berries not only for various food and beverage products, but also in cosmetics and for extraction of various biochemical compounds. The newly funded project “WILDBERRIES” (Norwegian Research Council) will focus on predictability of yield and quality of lingonberry (*Vaccinium vitis - idaea*). With characteristics like taste, secondary metabolites with health properties, versatility and preservative properties there is a great potential for value creation. It is estimated that the annual crop of lingonberries in Norway is 115,000 tones, most of it non-exploited. One of the key challenges for further commercialization is access to the raw material. The Norwegian topography are challenging for the logistic around harvesting. However, the same landscape can possibly give unique qualities. The availability and quality of wild berry yields vary from year to year and from locations to location. Yields are affected by climatic conditions years in advance, during the ripening and condition and management of the forest.

OBJECTIVE: WILDBERRIES aim to increase the commercial utilization of wild berries from Norwegian forests.

METHODS: WILDBERRIES seek to develop tools to map areas with high yields and/or high-quality berries. Experiments at controlled climatic conditions will give new knowledge on key factors affecting flower development, ripening, yield and quality.

RESULTS: Plots for phenotyping and berry collection will be established at different sites summer 2019. The existing clone collection of lingonberries will be increased, and controlled experiments will be performed from the second project year.

CONCLUSION: Wanted outcome of the project are models for prediction of yields and quality of the berries.

KEYWORDS: Lingonberries, modelling, *Vaccinium vitis - idaea*, quality

Effects of Red Raspberry Polyphenols and Metabolites on Biomarkers of Inflammation and Insulin Resistance in Pre-Diabetes and Type 2 Diabetes

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BACKGROUND: Diabetes is the seventh leading cause of death in the United States.

OBJECTIVE: To explore the efficacy of red raspberries (RR) (*Rubus idaeus*), as rich sources of polyphenolic compounds (PCs), and of its metabolites on modulation of biomarkers of inflammation and insulin resistance in diabetic and pre-diabetic patients.

METHODS: Anthocyanin profile was analyzed by HPLC-PDA-MS. Seven type 2 diabetes mellitus (T2DM) and two pre-diabetic patients were given one RR serving (123 g) per day as a smoothie for two weeks. Blood samples were drawn at baseline (BSL) and post-feeding (PF) days. The samples were analyzed for phenolic metabolites by UPLC-ESI-QTOF-MS/MS, and for both inflammation and insulin resistance biomarkers by a multiplex magnetic bead-based immunoassay. Insulin secretion using DOPAC (3,4-dihydroxyphenylacetic acid), a metabolite from anthocyanin and quercetin consumption in RR, was evaluated on INS-1 cells using ELISA.

RESULTS: Total anthocyanin concentration of RR extracts was 887.6 ± 262.8 $\mu\text{g/g}$ cyanidin-3-sophoroside (C3S) equivalents with C3S being the most prevalent. Two urolithin conjugates, i.e. urolithin A glucuronide (Uro-A glur) and urolithin A sulfate (Uro-A sulf) were found in 7 of the 9 patients' plasma samples at nanomolar concentrations on PF day whereas anthocyanin-derived metabolites such as protocatechuic acid (PCA) and DOPAC were present at higher but not statistically significant levels in both groups at PF day when compared to BSL. Results indicated significant reductions in hsCRP ($p=0.01$), and on insulin resistance where a statistical trend on HOMA-IR ($p=0.0584$) for T2DM patients was seen. The metabolite DOPAC when incubated at 1-100 μM did not stimulate insulin secretion in vitro. Increases and decreases were observed on the cytokines (IL-8, IL-10, IFN- γ , TNF- α , MCP-1, MIP-1, PAI-1, oxLDL) analyzed, yet, none was significant in either group.

CONCLUSION: This study demonstrates the potential of RR to modulate inflammation and insulin resistance in diabetic and pre-diabetic patients.

KEYWORDS: type 2 diabetes, pre-diabetes, red raspberries, polyphenols, metabolites, insulin resistance, inflammation.

Serum From Aged Humans Supplemented with Blueberry or Strawberry Reduces Inflammatory Stress Signals in Hapi Rat Microglial Cells, In Vitro

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BACKGROUND: Age-related decrements in cognition are thought to result from the increased susceptibility to and accumulating effects of oxidative stress and inflammation. Berry fruits contain a variety of bioactive polyphenolic compounds that exhibit potent antioxidant and anti-inflammatory activities. We have shown that consumption of freeze-dried whole berry powder, equivalent to 1 cup/day of blueberry (BB) or 2 cups/day of strawberry (SB), can differentially improve some aspects of cognition in healthy, older adults, compared to placebo-supplemented controls.

OBJECTIVE: Because the bioactive compounds in foods are different than those found in circulation following consumption, we were interested in whether pre-treatment of stressed cells with serum from people fed these foods may be a more valid model system than treating with extracts of the foods themselves for assessing their anti-inflammatory effects.

METHODS: We investigated whether fasting and postprandial serum from BB- or SB-supplemented older adults (60-75yo), taken at baseline or after 45 or 90 days of supplementation, would reduce the production of inflammatory and oxidative stress signals, as measured by western blot and ELISA techniques, compared to a placebo group, in LPS-stressed HAPI rat microglial cells, in vitro.

RESULTS: Serum from both blueberry and strawberry reduced nitrite production, iNOS and COX-2 expression and TNF α release relative to serum from placebo controls ($p < 0.05$). Protection was greatest with serum from the 90-day time-point, suggesting that ongoing supplementation may provide the most health benefits. Serum also showed protection in both fasted and postprandial conditions, suggesting that the meal did not challenge their ability to regulate oxidative and inflammatory stress.

CONCLUSIONS: These results suggest that berry metabolites, present in the circulating blood, may be mediating the anti-inflammatory effects of dietary berry fruit. It is likely that this attenuation of inflammation is responsible for the beneficial effects on age-related declines in cognition.

KEYWORDS: Inflammation, Oxidative stress, Antioxidants, Nitric Oxide, Anthocyanins

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Bile Acid Profiles in Human Biological Samples after Polyphenol Intake -- Preliminary Data

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BACKGROUND: Bile acids (BAs) play a critical role in regulating human health through the activation of BAs receptor farnesoid X receptor (FXR) and membrane G protein coupled bile acid receptor-1 (TGR5). Characterizing BAs in human biological samples after polyphenol intake will help guide investigations on the potential health effects of polyphenols via BAs metabolism.

OBJECTIVE: Develop methods to characterize BAs and their metabolites in human biological samples. Characterize changes in BAs profile after chronic polyphenol consumption.

METHODS: Plasma, fecal and urine samples from two human studies that included berry intake were used for developing qualitative analysis of BAs using UHPLC-QTOF. The compounds were identified based on the exact mass, fragmentation pattern, available reference standards and database search. To investigate the effect of chronic polyphenol consumption on BAs composition, pooled plasma samples (fasting and postprandial 2 h, n=6) from a chronic (45 and 90 days) strawberry supplementation study (24 g freeze dried/day) with an older population were analyzed.

RESULTS: Among 106 BAs and their metabolites which were tentatively identified in the samples used for method development, 70, 55, and 47 BAs species were characterized in plasma, feces and urine samples, respectively. The qualitative analysis of BAs in plasma from subjects following the strawberry consumption protocol detected 8 primary and 31 secondary BAs. After 90-days strawberry supplementation, two secondary BAs--glycolithocholic acid and 9(11), (5)-cholenic acid-3, 12-diol were decreased to undetectable levels in the pooled fasting sample and the FXR/ TGR5 agonists, including chenodeoxycholic acid, deoxycholic acid, cholic acid, glycodeoxycholic acid and taurocholic acid, showed increasing peak areas at 2 h postprandial compared to fasting.

CONCLUSIONS: The changes in BAs profiles in fasting and postprandial plasma samples after chronic strawberry feeding suggest that strawberry polyphenols may alter BAs metabolism and the FXR/TGR5 signaling.

KEYWORDS: Bile acids, polyphenol, UHPLC, human sample

Effect Of Red Raspberry (*Rubus Idaeus L.*) Consumption with or without Fructo-Oligosaccharide on Metabolic Indices: A Single-Blinded Randomized, Crossover Clinical Trial

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BACKGROUND: Red raspberries (RRB) contain a unique polyphenol profile associated with cardio-metabolic benefits. Fructo-oligosaccharides (FOS), as prebiotics, present an approach to augment the cardio-metabolic benefits of RRB through their known effects in modifying the gut microbiota composition.

OBJECTIVE: To determine the effect of RRB intake with or without FOS on glucose and insulin responses and fasting lipid profile in insulin-resistant (IR) (n = 20) and metabolically healthy reference (R) adults (n = 11) in a single-blinded, randomized, 12-week crossover clinical trial.

METHODS: In this crossover study, after one-week run-in, subjects consumed RRB (1 cup RRB equivalence) or RRB+FOS (1 cup RRB equivalence with 8g FOS) for 4 weeks in random order separated by 4-week washout between supplementation periods. Before and after each supplementation period, glucose and insulin responses were assessed by 2-h postprandial glycemic challenge with RRB (75 g glucose equivalence) and the plasma lipid profile characterized (total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG)).

RESULTS: IR group had significantly elevated fasting and postprandial glucose and insulin, and higher fasting TG and lower HDL compared to R at baseline (0-week) ($P \leq 0.05$); no differences in TC and LDL between groups ($P > 0.05$). After 4-week intervention, RRB decreased TC and LDL of IR group from baseline by 7% ($P = 0.024$) and 9% ($P = 0.007$), respectively, whereas adding FOS significantly attenuated the lipid-lowering effect of RRB. Alternatively, adding FOS to RRB augmented RRB effect on glycemic variables: 4-week intake of RRB+FOS significantly decreased 30 min incremental areas under curve (iAUC₀₋₃₀) of IR group for glucose and insulin from baseline by 20% ($P = 0.014$) and 18% ($P = 0.012$), respectively.

CONCLUSIONS: 4-week RRB intervention improves glycemic and lipid profiles in people with IR. Adding FOS to RRB supplement enhanced the glycemic benefits, but attenuated the lipid-lowering effect of RRB.

KEYWORDS: red raspberry; fructo-oligosaccharide; glycemic challenge; lipid profile; LDL

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