Project Title: Effects of Freeze Dried Strawberry Powder Supplementation on Vascular Function, Blood Markers of Cardiovascular Risk, and the Gut Microbiome

University of Arizona Collaborative Research Initiative with the California Strawberry Commission
Date: July 17, 2017

Type of Proposal: New
Amount of Funding Requested for 1st year: $117,336
Co-Principal Investigators: Ann C. Skulas-Ray and Chesney K. Richter
Organization: Department of Nutritional Sciences, The University of Arizona

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SUMMARY

Dietary interventions designed to reduce cardiovascular disease (CVD) risk by increasing consumption of particular health-promoting foods (e.g., strawberries) generally target blood pressure and LDL-C—the two endpoints recognized by the FDA for a cardiovascular health claim; however, CVD risk reduction may also be achieved via changes in emerging endpoints such as the gut microbiome. Previous research suggests that strawberries have the potential to reduce LDL-C, but it remains unclear whether there is a dose-response relationship. Moreover, few studies have evaluated effects on vascular health or characterized changes in the gut microbiome following daily strawberry consumption. Additionally, previous studies have largely been conducted among Caucasian populations. Given the demographics of the US, it is important to evaluate effects in study populations that include ethnicities that may have higher risk of type 2 diabetes and/or other CVD risk factors, such as Hispanics. We propose to examine the effects of 4 weeks of supplementation with two doses of freeze dried strawberry powder (low dose: 13 g/d and high dose: 40 g/d) on: 1) LDL-C and blood pressure; 2) gut microbiome profile; and 3) other CVD and type 2 diabetes risk factors, including glucose, insulin, and inflammatory markers. We will enroll overweight (BMI 25-36 kg/m^2) but otherwise healthy adults with moderately elevated LDL-C (>3.0 mmol/L) and/or prehypertension (120-159/80-99 mm Hg). This will optimize the potential for observing significant benefits on these outcomes. We will recruit 50 eligible participants with the expectation that at least 40 will complete the study. The placebo-controlled, crossover study design will allow for a direct comparison of dose-response within the same participant. We anticipate that the bioactive components of strawberries will reduce LDL-C and blood pressure, and modify the gut microbiome, with greater changes on the high dose. There is preliminary evidence that polyphenol-rich foods can modify gut microbiota profiles, but this would be the first study to characterize the effects of daily strawberry consumption. We are uniquely placed at the University of Arizona to enroll a larger percentage of Hispanic participants, who are often under-represented in clinical nutritional research. Results from the proposed study could strengthen the evidence for a cardiovascular health claim for strawberries and draw greater attention to strawberries as a phytonutrient-rich food that can lower LDL-C and blood pressure, and modify the gut microbiome.

JUSTIFICATION AND LITERATURE REVIEW

Current dietary recommendations to reduce CVD risk emphasize healthy dietary patterns that include specific health-promoting foods. This type of approach, focusing on whole foods and dietary patterns, is well-suited for broad public application and targets multiple CVD risk factors—including established endpoints such as LDL-C and blood pressure, as well as more novel outcomes such as the gut microbiome. Strawberries are likely to be effective for CVD risk reduction as they are a rich source of phytonutrients such as ellagic acid, anthocyanins, quercetin, catechin, and ascorbic acid. A 40 g dose of freeze dried strawberry powder provides a substantial amount of bioactive compounds with minimal calories. Doses of freeze dried strawberry powder up to 50 g/d have also been shown to be well-tolerated for 3-12 weeks [1, 2]. Previous evidence indicates that strawberries may be effective for lowering LDL-C and blood pressure [2, 3], and the phytonutrient profile of strawberries may also produce beneficial changes in the composition of the gut microbiome [4]; however, the health-promoting potential of strawberries remains underappreciated and additional evidence is needed.

With our colleagues at the Pennsylvania State University, we previously evaluated the postprandial effects of incorporating 40 g freeze dried strawberry powder into a high fat meal [5]. The design of this investigation was based largely on a previous study by Burton-Freeman et al. [6] in which incorporation of 10 g freeze dried strawberry powder in a moderate fat meal modestly attenuated peak triglyceride responses and LDL oxidation. We found no statistically significant differences in postprandial lipemia, glycemic responses, or vascular function following the strawberry meal versus the control meal [5]. We hypothesize that these disparate findings may be due, in part, to the fact that our study population was substantially younger and had much lower total cholesterol and LDL-C levels. Therefore, we now propose to study an older population (30-65 vs. 20-50 years old) with elevated LDL-C and/or blood pressure. At the University of Arizona, we will also be able to enroll a more diverse and at-risk population given the demographics of Tucson and the proximity and accessibility of one of our clinical research facilities to underserved Hispanic communities.

Longer-term supplementation (rather than an acute dose) may also be necessary to observe significant health benefits. Previous studies indicate that regular strawberry consumption likely has the potential to improve both lipids and lipoproteins, but additional research is needed to clarify these effects. For instance, beneficial effects on lipids and lipoproteins have been found with 3-12 weeks of strawberry consumption, but doses of freeze dried strawberry powder have ranged from 10 - 50 g/d [1, 2]. Additionally, little research has directly examined potential dose-response effects. To our knowledge, the only published dose-response study is specific to postprandial glucose and insulin responses following acute supplementation [7]. With regard to longer-term supplementation, a 50 g/d dose was shown to decrease LDL-C after 12 weeks in older adults with abdominal obesity and dyslipidemia [2], whereas 6 weeks of supplementation with 10 g/d did not significantly reduce LDL-C in overweight hyperlipidemic adults [6]. This suggests that a certain dose of strawberries may be required to achieve beneficial effects but a minimum required dose has not been definitively established. Only one study that we are aware of has examined the effect of long-term effect of two different doses on LDL-C within the same study population [2]. In this population of adults with abdominal adiposity and elevated lipids, 12 weeks of supplementation with either 25 g/d or 50 g/d of freeze dried strawberry powder reduced total cholesterol and LDL-C, with greater reductions achieved on the higher dose [2]. However, interpretation of these results is limited by the parallel-arm design of this study, and no

crossover dose-response studies have been published at this time. This type of study could aid in the determination of the amount of strawberries required to optimize effects on lipids/lipoproteins as it would not be confounded by potential between-subject differences at study entry.

With respect to blood pressure, there is evidence to indicate that berry consumption (including strawberries) can lower systolic blood pressure—particularly in individuals with higher baseline blood pressure values [8]. However, we are not aware of any published results regarding the effect of longer-term strawberry consumption on other measures of vascular health in adults with elevated CVD risk. Observational evidence suggests a protective relationship as greater anthocyanin and flavone intake, such as that achieved from 1-2 servings of berries per day, has been associated with lower central blood pressure and arterial stiffness [3]. However, intervention studies are needed to demonstrate a causal relationship and clarify how this relates to other CVD risk factors.

There is increasingly strong evidence that the gut microbiome plays a key role in health and disease, and may be a modifiable target for chronic disease risk reduction. To our knowledge, the composition of the gut microbiome following strawberry supplementation has yet to be characterized using state of the art sequencing technology. Previous studies have demonstrated that other polyphenol-rich plant-based foods such as blueberries can alter the microbiota profile and increase the abundance of beneficial, commensal bacteria [4, 9]. Strawberries are rich in the polyphenols ellagitannin and ellagic acid. These compounds are not readily absorbed but can be converted by gut microbiota to urolithins, which are more bioavailable and have been shown to have beneficial properties *in vitro* [10]. Thus, changes in the gut microbiome may be one of the mechanisms by which strawberry consumption can reduce CVD risk. We propose to use high-throughput sequencing technology to characterize changes in the composition of the gut microbiome following strawberry supplementation and determine whether strawberry polyphenols can effectively upregulate bacterial species associated with beneficial health outcomes.

OBJECTIVES

The proposed study will investigate whether sustained strawberry consumption can reduce LDL-C, lower blood pressure, and alter the gut microbiome. We will also evaluate whether strawberries can aid in the management of other cardiometabolic disease risk factors (e.g., fasting glucose and insulin, and inflammation). If results for these outcomes are significant, this will provide further evidence to support an FDA-approved CVD health claim for strawberries. This would encourage health professionals to recommend regular strawberry consumption as part of a healthy dietary pattern for CVD risk reduction.

Specific Aims

1. To determine whether 4 weeks of freeze dried strawberry consumption improves the lipid profile (primarily LDL-C, as well as total cholesterol, HDL-C, and triglycerides) and other CVD risk factors (i.e., glucose, insulin, and inflammatory markers).
2. To determine whether 4 weeks of freeze dried strawberry consumption improves vascular function, in terms of resting brachial and central blood pressure and arterial stiffness indices (i.e., augmentation index and pulse wave velocity).
3. To identify changes in the composition of the gut microbiome following 4 weeks of freeze dried strawberry consumption.

4. To determine whether there is a dose-response effect with greater improvements achieved following high dose (40 g/d) supplementation.

METHODS

Subjects & eligibility

For this study we will recruit overweight men and women (body mass index 25-36 kg/m^2) as this is representative of U.S. adults who are at higher CVD risk and might benefit the most from increased strawberry consumption.

Additional eligibility criteria will include:

- 30-65 years of age
- At least one of the following:
  - LDL-C above 3.0 mmol/L (116 mg/dL)
  - Systolic blood pressure of 120-159 mmHg
  - Diastolic blood pressure of 80-99 mmHg
- Total cholesterol below 6.2 mmol/L (240 mg/dL)
- Triglycerides below 350 mg/dL

Exclusion criteria will include:

- Allergies to strawberries
- History of CVD, Stage II hypertension (BP $\geq$ 160/100 mmHg), kidney disease, diabetes, or inflammatory diseases such as GI disorders and rheumatoid arthritis
- Use of medications/supplements for elevated lipids, blood pressure, or glucose
- Chronic use of non-steroidal anti-inflammatory or immunosuppressant drugs
- Conditions requiring chronic use of steroids

Experimental Design

We propose to conduct a 3-period randomized crossover dose-response study to evaluate the effect of freeze dried strawberries on LDL-C, vascular function, and the gut microbiome. Participants will be randomly assigned to three supplementation periods: 1) low-dose freeze dried strawberry powder (13 g/d); 2) high-dose freeze dried strawberry powder (40 g/d); and 3) a placebo powder. Each powder will be provided for 4-6 weeks, separated by a 2-week washout period/compliance break between treatment periods. Therefore, end of treatment values will be separated by at least 6 weeks (4-6 week supplementation period + 2 week break), which is sufficient for endpoints to stabilize. Total study time for participants will be approximately 16-20 weeks.

The high dose (40 g/d) will act as a proof of principle dose and is equivalent to the amount consumed acutely in our previous postprandial study. The 13 g/d low dose is equivalent to approximately 1 serving of fresh strawberries per day and represents a quantity that is easily achievable under free-living conditions. The use of both a low and high dose of freeze dried strawberry powder will allow us to investigate potential dose-response effects and determine whether benefits are achievable with smaller quantities. During each supplementation period, participants will be instructed to consume one package of study powder per day. Participants will be instructed to consume study powders in between meals and mixed with a water-based beverage to minimize the chance of other foods (e.g., dairy proteins) inhibiting absorption of strawberry bioactives. To maintain blinding and consistency in powder volume across

supplementation periods, freeze dried strawberry powder and placebo powder will be combined to achieve the low dose. For the proposed study population size (n = 50), this will require 2100 packages of each powder type (placebo, low-dose, and high-dose), provided in individually portioned packages, with the following contents:

- Placebo powder (40 g packages)
- Low-dose strawberry supplementation (40 g packages, consisting of 13 g freeze dried strawberry powder + 27 g placebo powder)
- High-dose strawberry supplementation (40 g packages, consisting of 40 g freeze dried strawberry powder)

We will recruit participants who consume a typical American diet (i.e. not vegetarian, not engaged in a structured diet plan, and not attempting to lose weight) to limit variability in endpoint responses. Participants will be instructed to keep their diet consistent and maintain body weight and physical activity levels during the study. Participants will also maintain a daily consumption log to monitor compliance and daily intake of study powders. Participants will receive an ~14 day supply of study powders on a biweekly basis, during which time study staff will monitor body weight and review consumption logs.

Testing visits

Clinical assessments (blood draw and vascular health measurements) will be conducted at baseline (prior to study start) and at the end of each of the three 4-week supplementation periods, on two consecutive days. Each day of testing will be performed following an overnight fast (12 hours with no food or drink except water). Testing will be performed at the University of Arizona Colloboratory Research Center and the Clinical and Translational Science Research Center (CATS). At each visit, participants will be weighed and their vital signs (temperature and blood pressure) will be measured. Vascular health measures (i.e., central BP and indices of arterial stiffness) will be performed on Day 1, prior to blood sampling. The fasting lipid profile will be measured on both days to account for natural variation in these values. Blood will be processed according to standard procedures for serum lipids and subsequent laboratory analysis of oxidative stress/inflammatory biomarkers (see following experimental endpoints).

Lipid profile and other blood markers of Cardiovascular Disease risk

Whole blood will be drawn into serum separator tubes, allowed to clot, and centrifuged. Total cholesterol and triglycerides will be determined by enzymatic procedures (Sonoran Quest). HDL-C will be estimated according to the modified heparin-manganese procedure (CV < 2%). LDL-C will be calculated using the Friedewald equation: LDL-C = TC – (HDL-C + TG/5) except in cases where triglyceride values exceed recommended ranges. In these cases a direct LDL-C assay will be done that measures LDL-C concentration using a chromogenic reaction after removal of all non-LDL-C (N-geneous LDL-ST-C, Sonoran Quest). The between run CV of this assay is less than 3%. Insulin will be measured by radioimmunoassay (Sonoran Quest). Glucose will be determined by Spectrophotometry procedures (Sonoran Quest).

Vascular function measures

Vascular function, in terms of central blood pressure and arterial stiffness indices, will be assessed using the SphygmoCor System pulse waveform analysis (AtCor Medical, Sydney, Australia). Measurements will be taken in a quiet, temperature-controlled, dimly lit room.

- **Pulse Wave Analysis (PWA): Aortic Blood Pressure and Augmentation Index**

Following a 5 minute seated rest, aortic (central) blood pressure and wave reflection characteristics (augmentation index) will be derived from brachial pressure waveforms using a validated generalized transfer function. On each test day, three PWA measures will be taken, following JNC 7 blood pressure guidelines, with 1 minute in between each reading. The last 2 PWA results will be averaged and used for analysis.

- **Pulse Wave Velocity (PWV): Aortic Stiffness**
  Aortic stiffness will be assessed by carotid-femoral pulse wave velocity (PWV). Carotid and femoral arterial pressure waveforms will be measured simultaneously via an applanation tonometry sensor manually held in place above the right common carotid artery and a blood pressure cuff placed on the right common femoral artery. PWV will subsequently be calculated by dividing the linear distance between the carotid and femoral sites by the transit time using the SphygmoCor system (AtCor Medical, Sydney Australia). On each test day, three PWV measurements will be obtained in the supine position, and the last two will be averaged for analysis.

Blood markers of inflammation
Serum hs-CRP will be measured by latex-enhanced immunonephelometry (Sonoran Quest).

Gut microbiome analysis
High-throughput sequencing of the 16S rRNA gene will be used to profile the bacterial community. This will identify and quantify dynamic changes in bacterial taxa present within the gut microbiome following supplementation. Stool samples will be collected at baseline and following each 4 supplementation period. Bacterial DNA will be extracted using the MoBio PowerFecal DNA Isolation Kit and the Qiagen Microbiome Kit will be used to eliminate host DNA. PCR amplification of the v3-v4 region of the 16S rRNA gene will be performed using the Illumina-tag PCR protocol. Purified library pools will be sequenced using Illumina MiSeq V3 500 cycle chemistry.

Statistical Analysis
Statistical analyses for blood markers of CVD risk, markers of inflammation, and vascular function measurements will be performed using SAS (version 9.4; SAS Institute). Treatment comparisons will be made between each of the supplementation periods. The mixed models procedure (PROC MIXED) will be used to test the effects of supplementation, period, and their interaction on outcome measures. Selection of model covariance structures will be based on optimizing fit statistics (evaluated as lowest Bayesian Information Criterion). Mixed models for endpoint means and change scores will be evaluated. Change scores will be calculated by subtracting baseline values from endpoint values. Means will be reported as least-squares means ± SEM. Baseline values will be included as covariates. For all tests, α will be set at 0.05.

For gut microbiome analyses, microbial DNA sequences will be quality filtered at an expected error of less than 0.5% using USEARCH v7. After quality filtering, reads will be analyzed using QIIME 1.9.0. An Operational Taxonomic Unit (OTU) table will then undergo cumulative sum scaling (CSS) normalization prior to beta diversity and alpha diversity analyses. Principal coordinates analysis (PCoA) plots and ANOSIM tests for significance will be performed using a weighted unifrac distance matrix. Linear Discriminant Analysis (LDA) effect size (LEfSe) will be used to identify potential differences in the microbial community following each intervention. Exploratory Spearman rank correlations will be performed between bacterial taxa and other physiological measurements (e.g., LDL-C, blood pressure, and inflammatory markers) to identify potential microbial signatures associated with CVD risk reduction.

The Principal investigator, Co-Principal Investigator and Authorizing Official for the University of Arizona, have reviewed and approved the proposal for consideration of funding.

7/17/17

Ann C. Skulas-Ray — Date

7/17/17

Chesney K. Richter — Date

for Kimberly Andrews Espy, Senior VP for Research — 7/26/17

Authorizing Official for the University of Arizona — Date

BUDGET Period: 2/1/18 - 1/31/19

Title of Proposal: Dose Response Effects of Freeze Dried Strawberry Powder Supplementation on Vascular Function, Blood Markers of Cardiovascular Risk, and the Gut

Key Personnel	Rate		1st Year
Ann Skulas-Ray, PhD	1%		
Salary Requested		$	805
Benefits (32.00%)		$	258
Chesney Richter, PhD	100%		
Salary Requested		$	47,660
Benefits (22.8%)		$	10,866
Other Personnel			
TBD Research Technician	25%		
Salary Requested		$	6,000
Benefits (32.00%)		$	1,920
Personnel Subtotal:		**$**	**67,509**
Equipment			
SphygmoCor System		$	30,000
Supplies and Materials			
Lab supplies		$	2,790
Equipment Maintenance		$	3,500
Subject pay (200 x 50)		$	2,000
Recruitment (advertising, etc.)		$	750
Screening fees/services		$	1,000
Screening Assays		$	1,000
Fees for Collaboratory space		$	2,667
Publication and Travel**			
Conference and Publication fees		$	-
Endpoint Analysis			
Lipids and Lipoproteins		$	1,000
Insulin and Glucose		$	1,600
Inflammatory Markers		$	-
Microbiome		$	-
Materials, Participants, Equipment and Publication		**$**	**46,307**
Yearly total		**$**	**113,816**

Strawberry Research Activities Report for previous funding

1. **Name of PI:** Ann C. Skulas-Ray and Penny M. Kris-Etherton (Co-PIs)
2. **Title of Project:** Effects of a freeze dried strawberry powder on postprandial vascular function and blood markers of cardiovascular risk
3. **Funding requested (for current and future years):** $0
4. **History of funding by Strawberry Commission:** $148,624 (2013-2015)
5. **Grant and funding support for strawberry research (not from the CSC):** None
6. **Research publications and presentations**
    a. **Referred publications:**
        - Richter CK, Skulas-Ray AC, Gaugler TL, Lambert JD, Proctor DN, Kris-Etherton, PM. Incorporating freeze-dried strawberry powder into a high-fat meal does not alter postprandial vascular function or blood markers of cardiovascular disease risk: a randomized controlled trial. *Am J Clin Nutr*. 2017. **105**(2): 313-322.

    b. **Non-referred scientific publications:** None
    c. **Presentations at scientific meetings (include date, location, and audience):** None
    d. **Leadership or participation in the organization of meetings, symposia, working groups, or other activities intended to discuss scientific topics relevant to strawberries and human health:** None
7. **Other presentations (California Strawberry Commission sponsored meetings with health professionals, food professionals, members of the media, etc.):** None

CURRENT FUNDING FOR DR. ANN C. SKULAS-RAY

Start-up package (including funds for laboratory supplies, personnel, and pilot studies) granted by the University of Arizona as part of hiring in November, 2015.

03/15- Present Omega Protein Corporation. *Gift of Unrestricted Funding.* $7,111. (Role: PI)

PREVIOUS FUNDING

*05/15-04/18 California Strawberry Commission. *Effects of Freeze Dried Strawberry Powder Supplementation on Vascular Function and Blood Markers of Cardiovascular Risk.* $299,366 (First year of funding: $99,988). (Role: Co-PI, PI: Penny Kris-Etherton)

*03/15-02/18 Ocean Spray Cranberries, Inc. *Effects of cranberry juice on cardiovascular risk factors in a placebo-controlled crossover trial.* $446,272. (Role: Co-PI, PI: Penny Kris-Etherton)

**Grants transferred to Dr. Penny Kris-Etherton after my hiring at the University of Arizona so that these in-progress clinical studies will be completed at the Pennsylvania State University.*

01/14-12/16 California Walnut Commission. *Effects of Walnuts on Cholesterol Lowering, Central Blood Pressure, Arterial Stiffness Indices, Lipoproteins, and other CVD Risk Factors.* $507,801 (Role: Co-PI, PI: Penny Kris-Etherton)

10/13-10/15 Pronova BioPharma. *Effects of a prescription omega-3 fatty acid concentrate in a placebo-controlled trial of human endotoxemia.* $250,000. (Role: Co-PI, PI: Gordon Jensen)

03/14-02/16 DuPont Nutrition & Health. *Effect of soy on HDL-C function, central blood pressure, and arterial stiffness.* $183,735. (Role: Co-I, PI: Penny Kris-Etherton)

04/13-04/15 California Strawberry Commission. *Effects of a Freeze Dried Strawberry Powder on Postprandial Vascular Function and Blood Markers of Cardiovascular Risk.* $148,264. (Role: Co-PI w/ Penny Kris-Etherton)

09/09-09/14 United States Department of Agriculture. *Dose response of omega-3 fatty acids in human endotoxemia: using a human model of induced inflammation for informing recommended intakes.* $500,000. (Role: Co-I, PI: Penny Kris-Etherton)

CURRICULUM VITAE
ANN CHRISTINE SKULAS-RAY, PHD

CHRONOLOGY OF EDUCATION

2014	Postdoctoral Fellow, The Pennsylvania State University, Department of Nutritional Sciences, University Park, PA Mentor: Penny Kris-Etherton, PhD, RD
2011	PhD, Nutrition, The Pennsylvania State University, University Park, PA
2005	BS, Biochemistry, Worcester Polytechnic Institute, Worcester, MA *With highest distinction*

CHRONOLOGY OF EMPLOYMENT

11/15-Present	Assistant Professor, The University of Arizona, Department of Nutritional Sciences, Tucson, AZ
09/14-11/15	Research Associate, The Pennsylvania State University, Department of Nutritional Sciences, University Park, PA
05/11-08/14	Postdoctoral Fellow, The Pennsylvania State University, Department of Nutritional Sciences
07/05-04/11	Graduate Fellow/Research Assistant, The Pennsylvania State University, University Park, PA
06/04-06/05	Research Fellow/Lab Assistant, University of Massachusetts Medical School, Worcester, MA

HONORS AND AWARDS

2014	**Baxter Postdoctoral Fellowship,** The Pennsylvania State University, University Park, PA
2011	**Travel award from the Eicosanoid Research Foundation**, to attend the Bioactive Lipids in Cancer, Inflammation, and Related Diseases conference in Seattle, WA
2011	**ASN Emerging Clinical Leaders Finalist**, American Society for Nutrition, Experimental Biology Conference. Washington, DC
2008	**Travel award from NIH**, to attend week-long Dietary Supplement Research Practicum on NIH campus, Office of Dietary Supplements, National Institutes of Health.
2006	**Huck Institutes of the Life Sciences Fellowship**, The Pennsylvania State University. University Park, PA
2005	**Barry M. Goldwater Scholarship**, Worcester Polytechnic Institute, Worcester, MA

PUBLICATIONS

Refereed Journal Articles

1. Parker J, Atez F, Rossetti RG, **Skulas A**, Patel R, Zurier RB. Suppression of human macrophage interleukin-6 by a nonpsychoactive cannabinoid acid. *Rheumatol Int.* 2008;28(7):631-5.
2. **Skulas-Ray AC,** West SG, Davidson MH, Kris-Etherton PM. Omega-3 fatty acid concentrates in the treatment of moderate hypertriglyceridemia. *Expert Opin Pharmacother.* 2008;9(7):1237-48. [Also published in Spanish]

3. Zurier RB, Sun YP, George KL, Stebulis JA, Rossetti RG, **Skulas A**, Judge E, Serhan CN. Ajulemic acid, a synthetic cannabinoid, increases formation of the endogenous proresolving and anti-inflammatory eicosanoid, lipoxin A4. *FASEB J.* 2009;23(5):1503-9.
4. **Skulas-Ray AC**, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr.* 2011;93(2):243-52. [Faculty of 1000 selection, 4th most viewed article published in 2011]
5. **Skulas-Ray AC,** Kris-Etherton PM, Teeter DL, Chen CY, Vanden Heuvel JP, West SG. A high antioxidant spice blend attenuates postprandial insulin and triglyceride responses and increases some plasma measures of antioxidant activity in healthy, overweight men. *J Nutr.* 2011;141(8):1451-7.
6. Sauder KA, Johnston ER, **Skulas-Ray AC**, Campbell TS, and West SG. Effect of meal content on heart rate variability and cardiovascular reactivity to mental stress. *Psychophysiology.* 2012;49(4):470-7.
7. McCrea C, **Skulas-Ray AC**, Chow M, West SG. Test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. *Vasc Med.* 2012;17(1):29-36.
8. **Skulas-Ray AC**, Kris-Etherton PM, Harris WS, West SG. Effects of marine-derived omega-3 fatty acids on systemic hemodynamics at rest and during stress: a dose-response study. *Ann Behav Med.* 2012;44(3):301–308.
9. Sauder KA, **Skulas-Ray AC**, Campbell TS, Johnson JA, Kris-Etherton PM, West SG. Effects of omega-3 fatty acid supplementation on heart rate variability at rest and during acute stress in adults with moderate hypertriglyceridemia. *Psychosomatic Med.* 2013;75(4):382-9.
10. Flock MR, **Skulas-Ray AC**, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial. *Journal of the American Heart Association.* 2013;2(6): e000513.
11. Blumberg J, Camesano T, Cassidy A, Kris-Etherton P, Howell A, Manach C, Ostertag L, Sies H, **Skulas-Ray A**, Vita J. Cranberries and Their Bioactive Constituents in Human Health. *Advances in Nutrition.* 2013;4(6):618-32.
12. West SG, Crispell MD, Piotrowski MJ, Poupin N, Miller DL, Preston AG, Wagner P, Groves LF, and **Skulas-Ray AC**. Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults. *British Journal of Nutrition.* 2013;25:1-9.
13. Flock MR, **Skulas-Ray AC**, Harris WS, Gaugler TL, Fleming JA, and Kris-Etherton PM. Effects of supplemental long-chain omega-3 fatty acids and erythrocyte membrane fatty acid content on circulating inflammatory markers in a randomized controlled trial of healthy adults. *Prostaglandins, Leukotrienes, and Essential Fatty Acids.* 2014;91(4):161-168.
14. Richter CK, **Skulas-Ray AC**, and Kris-Etherton PM. Recent findings of studies on the Mediterranean Diet: What are the implications for current dietary recommendations? *Endocrinology and Metabolism Clinics North America* 2014;43(4):963–980.
15. Kim JK, Moore DJ, Maurer DG, Kim-Shapiro DB, Basu S, Flanagan MP**, Skulas-Ray A**, Kris-Etherton, P and Proctor DN. Acute dietary nitrate supplementation does not augment submaximal forearm exercise hyperemia in healthy young men. *Applied Physiology, Nutrition, and Metabolism.* 2015;40(2):122-8.
16. **Skulas-Ray AC**, Alaupovic P, Kris-Etherton PM, West SG. Dose Response Effects of Marine Omega-3 Fatty Acids on Apolipoproteins, Apolipoprotein-Defined Lipoprotein Subclasses, and Lp-PLA_2 in Individuals with Moderate Hypertriglyceridemia. *J Clin Lipidol.* 2015;9(3):360-367.

17. McCrea CE, West SG, Kris-Etherton PM, Lambert JD, Gaugler TL, Teeter DL, Sauder KA, Gu Y, Glisan SL, and **Skulas-Ray AC**. Effects of culinary spices and psychological stress on postprandial lipemia and lipase activity: results of a randomized crossover study and *in vitro* experiments. *Journal of Translational Medicine.* 2015;13(7):1-12.
18. **Skulas-Ray AC**. Omega-3 Fatty Acids and Inflammation: A Perspective on the Challenges of Evaluating Efficacy in Clinical Research. *Prostaglandins & Other Lipid Mediators.* 2015;116-117C:104-111
19. **Skulas-Ray AC**, Flock MR, Richter CK, Harris WS, West SG, Kris-Etherton PM. Red Blood Cell Docosapentaenoic Acid (DPA n-3) is Inversely Associated with Triglycerides and C-Reactive Protein (CRP) in Healthy Adults and Dose-Dependently Increases Following n-3 Fatty Acid Supplementation. *Nutrients.* 2015;7:6390-6404.
20. Richter CK, **Skulas-Ray AC**, Champagne C, Kris-Etherton PM. Plant protein and animal protein: do they differentially affect cardiovascular risk? *Advances in Nutrition.* 2015;6(6): 712-28.
21. Richter CK, **Skulas-Ray AC**, Gaugler TL, Lambert JD, Proctor DN, Kris-Etherton, PM. Incorporating freeze-dried strawberry powder into a high-fat meal does not alter postprandial vascular function or blood markers of cardiovascular disease risk: a randomized controlled trial. *Am J Clin Nutr.* 2017. **105**(2): 313-322.
22. Richter CK, **Skulas-Ray AC**, Fleming JA, Link CJ, Mukherjea R, Krul ES, Kris-Etherton PM. Effects of isoflavone-containing soya protein on ex vivo cholesterol efflux, vascular function and blood markers of CVD risk in adults with moderately elevated blood pressure: a dose–response randomised controlled trial. *British Journal of Nutrition* 117.10 (2017): 1403-1413.
23. Richter CK, Bowen KJ, Mozaffarian D, Kris-Etherton PM, **Skulas-Ray AC**. Omega-3 Fatty Acid Intake and Food Sources in the United States Compared to Recommended Intakes: NHANES 2003-2008. *Lipids.* Accepted pending minor revisions.

Book Chapters

1. **Skulas-Ray AC**, Flock M, Kris-Etherton PM. The role of diet in the prevention and treatment of cardiovascular disease. In: Coulston AM, Boushey CJ, Ferruzzi M, eds. *Nutrition in the Prevention and Treatment of Disease.* 3rd ed. Waltham, MA: Academic Press; 2012.
2. **Skulas-Ray AC**, Fleming JA, and Kris-Etherton PM. Dietary Patterns for the Prevention and Treatment of Cardiovascular Disease. In Ballantyne, CM, ed. *Clinical Lipidology: A Companion to Braunwald's Heart Disease.* 2nd ed. Philadelphia, PA: Elsevier; 2014.
3. Richter CK, **Skulas-Ray AC**, and Kris-Etherton PM. Recommended Intake of Fish and Fish Oils Worldwide. In Raatz, S and Bibus, D eds. *Fish and Fish Oils in Health and Disease.* San Diego, CA: Elsevier; 2016.
4. Richter CK, **Skulas-Ray AC**, and Kris-Etherton PM. The role of diet in the prevention and treatment of cardiovascular disease. In: Coulston AM, Boushey CJ, Ferruzzi M, eds. *Nutrition in the Prevention and Treatment of Disease.* 4th ed. Waltham, MA: Academic Press, 2017.

Encyclopedia Entries

1. **Skulas-Ray AC**. Resolution of inflammation. In: MacKay I, Rose NR, eds. *Encyclopedia of Medical Immunology*. New York, NY: Springer; 2014.
2. Kris-Etherton PM, Fleming J, Kroat A, **Skulas-Ray A**, and Flock M. The Role of Nutrition in Heart Disease Prevention. In: Dulbecco R, ed. *Encyclopedia of Human Biology, 3rd Ed.* Oxford, UK: Elsevier; 2015.

CURRICULUM VITAE
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Education

2013-2016 Dual-title PhD in Nutritional Sciences and Clinical & Translational Science, The Pennsylvania State University
Dissertation: *Plant-derived Bioactives and Long-Chain Omega-3 Fatty Acids as Nutritional Interventions for Cardiovascular Disease Risk Factors*

2008-2013 B.S. in Biology with Honors, Juniata College, Huntingdon, PA
Cumulative GPA: 3.976
Undergraduate thesis: *Analysis of Cumin Spice using Laser-Induced Breakdown Spectroscopy (LIBS)*

Research Experience

2014-Present **Effects of a prescription omega-3 fatty acid concentrate in a placebo-controlled trial of human endotoxemia**: Coordinated clinical study investigating the effects of EPA+DHA supplementation (3.4 g/d) on inflammatory responses (i.e., C-reactive protein, tumor necrosis factor-α, and interleukin-6), oxidative stress (i.e., urinary isoprostanes), and pro-resolving lipid mediator production (e.g., resolvins, protectins, and maresins) following induced endotoxemia.
Graduate Student Study Coordinator, The Pennsylvania State University
Advisor: Dr. Penny Kris-Etherton

2013-2016 **Effects of a Freeze Dried Strawberry Powder on Postprandial Vascular Function and Blood Markers of Cardiovascular Risk**: Coordinated clinical study investigating the effects of plant-based bioactives on postprandial vascular function (i.e., central blood pressure, augmentation index, and pulse wave velocity), as well as triglycerides, glucose, and insulin responses following the consumption of a high-fat meal.
Graduate Student Study Coordinator, The Pennsylvania State University
Advisor: Dr. Penny Kris-Etherton

2014-2015 **Effects of Walnuts on Central Blood Pressure, Arterial Stiffness Indices, Lipoproteins, and other CVD Risk Factors**: Launched controlled feeding study investigating the effects of bioactives and alpha-linolenic acid in walnuts and trained incoming graduate student to coordinate study.
Graduate Student Study Coordinator, The Pennsylvania State University
Advisor: Dr. Penny Kris-Etherton

2012-2013 **Analysis of Cumin Spice using Laser-Induced Breakdown Spectroscopy (LIBS)**: Developed a model to verify the geographic origin of cumin spice based on its elemental composition.
Undergraduate Research Assistant, Juniata College, Chemistry
Advisor: Dr. Richard Hark

2012-2013 **Modulation of the human gut microbiome in response to resistant starch diets**: Performed statistical analysis of 16S gut microbiome data from a clinical study investigating the effect of diets containing varying amounts of resistant starch, using Quantitative Insights into Microbial Ecology (QIIME).
Undergraduate Research Assistant, Juniata College, Biology
Advisor: Dr. Regina Lamendella

Summer 2012 **Analysis of Phytochemical Content in Biscuits Containing Varying Amounts of Whole Wheat and Fiber**: Extracted free and bound phenolics from samples and analyzed total phenolic contents using the Folin-Ciocalteu method and spectrophotometer analysis.
Undergraduate Summer Research Scholar, Cornell University
Advisor: Rui Hai Liu

Peer-reviewed publications

1. **Richter CK**, Skulas-Ray AC, Fleming JA, Link CJ, Mukherjea R, Krus ES, Kris-Etherton PM. Effects of isoflavone-containing soya protein on ex vivo cholesterol efflux, vascular function and blood markers of CVD risk in adults with moderately elevated blood pressure: a dose–response randomised controlled trial." *British Journal of Nutrition* 117.10 (2017): 1403-1413.
2. **Richter CK**, Skulas-Ray AC, Gaugler TL, Lambert JD, Proctor DN, Kris-Etherton PM. Incorporating freeze-dried strawberry powder into a high-fat meal does not alter postprandial vascular function or blood markers of cardiovascular disease risk: a randomized controlled trial. *The American Journal of Clinical Nutrition* 105.2 (2017): 313-322.
3. Schieffer KM, Peters DG, **Richter CK**, Loc WS, Pawelczyk JA. Incorporating Informatics for Integrating Biology and the Bedside (i2b2) into Pre-doctoral Trainee Curriculum to Evaluate Student-Generated Hypotheses. *Clin Transl Sci* 2015;8(6): 729-33.
4. **Richter CK**, Skulas-Ray AC, Champagne CM, Kris-Etherton PM. Plant protein and animal protein: do they differentially affect cardiovascular disease (CVD) risk? *Adv Nutr* 2015;6(6):712-28.
5. Skulas-Ray A, Flock M, **Richter C**, Harris W, West S, Kris-Etherton P. Red Blood Cell Docosapentaenoic Acid (DPA n-3) is Inversely Associated with Triglycerides and C-reactive Protein (CRP) in Healthy Adults and Dose-Dependently Increases Following n-3 Fatty Acid Supplementation. *Nutrients* 2015;7(8):5291.
6. **Richter CK**, Skulas-Ray AC, Kris-Etherton PM. Recent findings of studies on the Mediterranean diet: what are the implications for current dietary recommendations? *Endocrinol Metab Clin North Am* 2014;43(4):963-80.

Manuscripts under review

1. **Richter CK**, Skulas-Ray AC, Bowen KJ, Mozaffarian D, Kris-Etherton PM. Omega-3 Fatty Acid Intake and Food Sources in the United States Compared to Recommended Intakes: NHANES 2003-2008. *Lipids* (accepted pending minor revisions)

Manuscripts in preparation

1. Skulas-Ray AC*, **Richter CK***, Jensen GL, Fleming JA, Flock ML, Kris-Etherton PM. Pilot study of the extended time course of inflammatory and metabolic responses over a 5 day period following low-dose intravenous endotoxin administration in health young men.

Book chapters

1. **Richter CK**, Skulas-Ray AC, Kris-Etherton PM. (2016). Recommended Intake of Fish and Fish Oils Worldwide. In: Raatz S and Bibus D (eds.), *Fish and Fish Oils in Health and Disease* (pp. 27-48). San Diego, CA: Academic Press
2. **Richter CK**, Skulas-Ray AC, Flock MR, Kris-Etherton PM. (2017) The Role of Diet in the Prevention and Treatment of Cardiovascular Disease. In: Boushey CJ, Coulston AM, et al. (eds.) *Nutrition in the Prevention and Treatment of Disease* (4th ed.) (pp. 595-615). Waltham, MA: Academic Press.

Scholarships and Awards

2014-2016	**National Institutes of Health Clinical and Translational Sciences Institute TL1 Career Development Award**, The Pennsylvania State University. *Awarded to students enrolled as a dual-title PhD in Clinical and Translational Sciences, demonstrating overall scholarly excellence, clearly delineated career research goals, and strong commitment to interdisciplinary research.*
2015	**Grace M. Henderson Graduate Scholarship**, The Pennsylvania State University. *Awarded to students who exemplify the qualities of a scholar, show professional promise and a dedicated interest in human service.*
2013-2014	**University Graduate Fellowship**, The Pennsylvania State University. *Awarded to one hundred incoming graduate students based on academic quality.*

Conference Presentations

2014 Winter Eicosanoids Conference (Poster Presentation) *In healthy adults supplemented with marine omega-3 fatty acids total isoprostane response to low-dose endotoxin challenge is attenuated despite increased f_3-isoprostane production.* **Richter CK**, Skulas-Ray AC, Milne G, Flock MR, Kris-Etherton PM.

Teaching Experience

Spring, 2014	Teaching Assistant for Nutrient Metabolism II (NUTR 446, 3 credit hours) Instructor: Vijay Kumar

Professional and Service Activities

2016-present	Reviewer, British Journal of Nutrition
2015-present	Reviewer, Nutrition Journal
2015-present	Reviewer, Lipids in Health and Disease
2016-present	Member, American Heart Association
2015-present	Member, American Society for Nutrition
2015-present	Member, Kappa Omicron Nu Honor Society