Supporting Local Agriculture via Clinical Research: Human Studies with Elderberries to Improve Biomarkers of Obesity

Progress report for ONE19-350

Project Type: Partnership
Funds awarded in 2019: $29,998.00
Projected End Date: 11/30/2022
Grant Recipient: University of Vermont
Region: Northeast
State: Vermont
Project Leader:

Dr. Patrick Solverson
Washington State University

Project Information

Project Objectives:

This project seeks to discover the health promoting effects of elderberries in humans.

Specifically, this project aims to determine the anti-obesity and anti-diabetic effects of elderberries when fed in a food (not extract) form. We have previously tested these effects in other berries and have observed increases in both fat oxidation and insulin sensitivity in overweight subjects after one week of feeding. Elderberries are a rich source of the bioactive components of berries thought to elicit positive effects on these measures, thus if a threshold dose of the bioactive compounds is necessary in order to see the benefit to both fat oxidation and insulin sensitivity, elderberries will allow the consumer to achieve these doses more efficiently than other berries. A recent project by the University of Vermont (UVM) identified elderberries as an agriculturally productive buffer crop; the plant is hearty and can be planted in the buffer strip between watersheds and conventional crops. If our project can demonstrate improvements in fat oxidation and insulin sensitivity in a human feeding study, elderberry-producing Vermont farmers can use the study as a marketing tool to generate interest in consumers, while assisting farmers in meeting the requirements of the clean water act of Vermont.

Introduction:

The Vermont clean water act was passed in 2015. An innovative solution to assist farmers to meet the requirements of the act was the investigation into elderberries as an “agriculturally productive buffer crop”, meaning farmers could reduce the runoff associated with their main cropland by strengthening the buffer strip with a crop that could also provide added revenue while meeting Vermont’s required agricultural practices. The University of Vermont center for sustainable agriculture

composed a 102-page growing guide as a tool to assist farmers who may be interested in augmenting their growing operation with the elderberry buffer strip, as well as a pricing tool that would estimate the up-front costs and subsequent, yield-based profits. Fortuitously, elderberries present a remarkably high anthocyanin content. Recent work has described the potential for anthocyanin containing berries to prevent obesity and type-2 diabetes in rodent models, and human studies also demonstrate beneficial effects of berry consumption on insulin sensitivity and fat oxidation. As elderberries are rich in the plant components thought to improve human health, this grant application proposes conducting a human feeding study with elderberries, as positive findings could be used as an innovative approach to market the crop. Positive findings from the proposed study would drive a higher demand for elderberry products, thus attracting interested farmers to plant the crop in their buffer strips, which would sequester more runoff attributable to conventional farmland and ultimately create healthier watersheds in Vermont. Successful implementation of the project and downstream interest in the state could be a model for other Northeastern states, as the elderberry varieties developed in Vermont are robust to Northeast growing conditions.

An added benefit of the proposed work would be the improved health of the local community if elderberry products were to become more available; two-thirds of all Americans struggle with excess body weight caused by poor diet choices and lack of physical activity. Obesity is taxing the healthcare system an estimated 9.1% of all annual medical spending, with a majority of expenditures going towards prescription drugs that treat the symptoms of obesity, not the cause. Performing work that expands the demand and production of elderberries is by no means a cure-all for the complex issue of obesity, but growing commodities that are healthful and readily available to the community rekindles a reciprocity between grower and consumer that promotes local farm operations in an era of rapid socio-economic change that presents ever-evolving challenges to the practice. The demonstration by the farming community to not only meet the new regulations of the Vermont clean water act, but to do so with an approach that generates a nutritious product will strengthen the bond between farmer and consumer while creating another market to help local farmers thrive.

The proposed work would establish a working relationship between the PI and the local growers. The combination agronomic and clinical research of future work would be a novel partnership for product advertisement based on the improvement of clinical variables from human studies.

Summer 2020 update: The PI has accepted a position at Washington State University's School of Medicine in an effort to streamline nutrition research as it relates to commodity agriculture. The PI's new position is well suited to continue the clinical trial portion of this proposed work and aims to bring the project with to his new appointment, where he has received the support of his new home department to do so.

January 2021 update: Clinical research is under an indefinite moratorium due to the COVID-19 pandemic. The PI has submitted the study to WSU IRB for approval of the clinical trial and anticipates a timely transition into study commencement once the moratorium is lifted.

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Research

Materials and methods:

Introduction/Update: In October of 2019 we purchased 218 pounds of elderberries from our partner farmers following the fall harvest. 218 pounds is below our target of 400 pounds, but we can likely obtain other berries from producers connected with a collaborator in Missouri and North Carolina. The 218 pounds of berries are in frozen storage while we work on method development described in SA1; we are currently still operating on SA1. I am working with a chemist on campus at UVM to create an acceptable method to quantify both cyanogenic glycosides as well as the primary anthocyanin in elderberries, cyanidin-3-glucoside. Based on the review of a position statement from a respected group of Nutritional Biochemists at NC State who specialize in anthocyanin quantification, we have decided to move away from the simpler, and less reliable, plate-based spectrophotometric assay for anthocyanin quantitation and instead are employing triple-quad mass spectrometry for determination of all of our compounds. This increases the quality of our data as well as the likelihood of publishing our work in respectable journals. Developing a reliable method to quantify our compounds is pivotal to the success of this project. We are attempting to reproduce an extraction method shared with us by a group at the University of Missouri. Once we have an effective analytical method in place we will begin to conduct cooking experiments to determine effects on our compounds. We are also in the process of obtaining IRB approval from UVM to conduct the clinical trial in order to address SAs 2-3.

The primary use of grant funds will be securing elderberries from local farmers in order to conduct a human feeding study to investigate their health promoting effects, specifically on the ability to augment nutritional approaches to combat obesity and diabetes. The elderberry treatment will require a standardized preparation (cooking method) that removes a plant metabolite (sambunigrin) without drastically reducing polyphenol content before the treatment can be fed to human subjects. Thus, there are three specific aims (SA) that comprise this

elderberry feeding project:

1. Standardize an elderberry cooking method, using conventional kitchen appliances, that successfully removes sambunigrin while preserving polyphenol content.
2. Perform a randomized, placebo controlled human feeding study that feeds the developed elderberry product to overweight and obese human volunteers for a period of 1 week to determine changes on fat oxidation using indirect calorimetry.
3. Perform a randomized, placebo controlled human feeding study that feeds the developed elderberry product to overweight and obese human volunteers for a period of 1 week to determine influence on insulin sensitivity using a meal tolerance test.

SA1

Elderberries contain trace amounts of sambunigrin that can be inactivated with cooking. Although the cooking step is a simple and necessary approach to remove the trace toxin, the concern is the preservation of the parent polyphenol compounds (anthocyanins are a subclass of flavonoids, a type of polyphenol) that are thought to explain the bioactive effects of berries with regard to the augmentation of fat oxidation and insulin sensitivity. We intend to study the effects of temperature and time on elderberry preparation in order to develop an optimal protocol that successfully removes sambunigrin while preserving polyphenol content for use in our human feeding study. Sambunigrin can be quantified using liquid chromatography-mass spectrometry as described previously. The total polyphenol content can be measured using a validated, plate-based spectrophotometric assay. A factorial design will be implemented, manipulating both cooking time and temperature, including two to three levels within each variable to generate a possible six to nine treatment groups in order to identify the group that yields the best conservation of polyphenols while eliminating trace sambunigrin. Treatment groups will be tested for differences in sambunigrin and polyphenol content using two-way ANCOVA with SAS version 9.4 including time, temperature, and interaction terms as fixed effects. Mass of starting materials (grams of berries and grams of water) will be included in the model statement as covariates to determine possible effects on both dependent variables.

SA2

Once an acceptable elderberry product has been developed, it will be tested for the ability to augment fat oxidation in overweight and obese human subjects enrolled in a feeding study. The feeding study will utilize a randomized, placebo-controlled, cross-over design. Proximate analysis (performed by Covance laboratories) of the elderberry product will allow for the creation of a polyphenol-free placebo food that matches the caloric and carbohydrate content of the berry treatment, which in past research was a gelatin food. This initial study will feed subjects for 7 days of each treatment (separated by a 28-day washout period) before performing indirect calorimetry measurements to assess potential differences in fat oxidation. The study will be powered to detect significant differences in the respiratory quotient between the two treatments. Our earlier study reached 80% power with the inclusion of 15 male subjects. Thus, because of the unknown potential differences between males and females, 18 men and 18 women will be enrolled in this study. The three additional subjects in each sex is to account for an anticipated 20% attrition rate, which is common in clinical research.

Indirect calorimetry is the measurement of respiratory gases in animals (the

volumes of carbon dioxide production and oxygen consumption) which is a relative indication of which macronutrient is oxidized for energy production, as calculated by the respiratory quotient (CO_2 produced/O_2 consumed). Further calculations will estimate grams of fat and carbohydrate oxidized for energy as well as energy expenditure. Respiratory gases can be measured using whole-room calorimetry, or with metabolic carts. The clinical research center (CRC) at the UVM is equipped with metabolic carts and the PI and graduate student will perform respirometry measurements at the end of each diet period. Interestingly, we previously reported significant increases in fat oxidation when subjects performed moderate-intensity physical activity during a berry treatment. Others have also reported similar findings. Thus, indirect calorimetry will be measured while the subject is at rest, and during a bout of low-to-moderate physical activity, to address potential augmentation of fat oxidation via elderberry consumption. Treatment differences in indirect calorimetry measurements (respiratory quotient, grams of fat and carbohydrate oxidation, and energy expenditure) will be tested for statistical significance via repeated-measures linear mixed models ANCOVA using the “Proc Mixed” function in SAS 9.4. Each dependent variable will be tested for main effects of treatment (elderberries or gelatin), sex, and treatment sequence. Subject age and BMI will be included as covariates and 2-way interaction terms of treatment with the other main effects and covariates will be investigated.

SA3

In addition to indirect calorimetry we will investigate the insulin sensitizing potential of elderberry consumption with the administration of an MTT. The oral glucose tolerance test (OGTT) is a common clinical tool used to assess glucose tolerance in human subjects. Similarly, the MTT has demonstrated construct validity with the OGTT, and is a more physiologic assessment of glucose tolerance and insulin sensitivity. The MTT is an attractive tool for the clinical nutritionist because it allows for the investigation of the acute (post-meal) effects of the food of interest on glucose tolerance and insulin sensitivity. Therefore, we will administer an MTT to the subjects at the end of each diet period, comprised of the treatment food, waffles, and syrup in order to deliver 75 grams of glucose. Blood collections will take place every 30 minutes out to three hours from the start of the meal in order to quantify blood glucose and insulin using a clinical chemistry analyzer and an ELISA assay, respectively.

June 2020 UPDATE: For two reasons we will no longer consider the work described in specific aim 1 and instead will focus on specific aims 2 and 3, which was and remains the central focus of this project. First, our preliminary work in collaboration with Bruce O'Rourke, chemist at UVM employing the triple-quadruple mass spectrometry techniques to measure sambunigrin and anthocyanins, detected negligible amounts of sambunigrin and amygdalin (a second cyanogenic glycoside also measured in elderberries). If we had the opportunity to perform the cooking experiment before the COVID-19 shutdown, we would have had to spike external standards into elderberry juice in order to achieve a meaningful signal on the MS for quantification after the cooking experiments. Further, this non-detection of cyanogenic glycosides is corroborated by Dr. Michael Greenlief's group at the University of Missouri. Through personal communication, they report that raw elderberry juice contains lower levels of cyanogenic glycosides compared to commercially available, store-bought apple juice. We will cite their work once it is published. Second, UVM's institutional review board mandated that we procure commercially available elderberry juice and would not permit us to feed the elderberry juice we procured through our partner farmers. Fortunately, we identified Terry Durham, owner of River Hill's Harvest in central Missouri, who agreed to sell us 240 11-ounce bottles (20 cases) of 100% elderberry juice from the same lot from

their 2019 Harvest. Dr. Penelope Perkins-Veazie kindly performed an anthocyanin analysis of their elderberry juice and verified that the study volunteers in our prospective clinical trial will achieve a 360 mg dose of cyanidin-3-glucoside equivalents with a half-cup of elderberry juice per day. We have purchased an adequate supply from Terry Durham to allow us to feed 36 subjects for 7 days as originally described.

We have no intention of letting the elderberry juice procured from our partner farmers go to waste. We are shipping the juice frozen to the PI's new appointment at WSU for use in cell culture experimentation related to cellular level energetics at a later date. All work involving those materials will cite this grant's support.

The PI's home department (Nutrition and Exercise Physiology) is in support of conducting specific aims 2-3 with the River Hill's Harvest elderberry juice. The juice is in sealed glass containers, where the cardboard boxes experienced significant deterioration during shipment from Missouri to Burlington, VT. As a precaution, the PI is transporting the elderberry juice in his personal vehicle out to WSU this summer to avoid further shipping damages.

January 2021 Update: PI and elderberry juice containers arrived safely in Spokane, Washington this past summer. PI is working with NC State's Food Innovation Laboratory to develop a placebo control in the hopes of double-blinding the study. Study details have been submitted to WSU IRB for approval of the clinical trial. PI anticipates an efficient initiation of the clinical trial once the COVID-19 moratorium on in-person research is lifted at a to-be-determined date, hopefully sometime during the 2021 calendar year.

Participation Summary

2 Farmers participating in research