Watermelon Focused Dietary Inflammatory Index Intervention

Clinical Trials Registration: NCT03158740

04/2017

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IRB Protocol
April, 2017

In addition to the information provided in this protocol, the original research proposal will be included with the IRB application. The focus of this IRB application is on intervention participants.

Project Summary:

Chronic inflammation underpins the development of numerous chronic diseases that contribute significantly to disability and mortality in the United States (US).[1,2] Of particular interest, as it relates to inflammation, is diet which is a strong moderator of systemic inflammation.[3,4] Diets high in fruit and vegetable intake, fish, and whole grains (e.g., Mediterranean and vegetarian diets) have been associated with lower levels of inflammation.[5,6] On the other hand, diets rich in red meat, high-fat dairy products, and refined grains (Western diet) are typically associated with higher levels of inflammation.[7,8] Up until 2014, no dietary index has been designed specifically to determine the inflammatory potential of individual's diet. However, researchers in the Cancer Prevention and Control Program, University of South Carolina developed the Dietary Inflammatory Index™ (DII).[9] The DII quantifies the level of dietary inflammatory potential on a scale from maximally anti- to pro-inflammatory. Watermelon is one of the fruits whose components have been shown to have anti-inflammatory effects.[10] The overall objective of this proposal is to determine if a refined and modified watermelon-focused DII counselling system can reduce levels of chronic inflammation. The DII is a validated measure that has been shown to predict a range of outcomes including inflammation, cancer, CVD, and mortality, among others.[11-19]
Specifically, the aims of this proposal are to:

1. Refine and modify the DII-based intervention by developing and incorporating numerous watermelon recipes which will score low (anti-inflammatory) on the DII scale.
2. Design and implement a two-arm intervention trial based on the refined watermelon-focused DII-based program for reducing levels of chronic inflammation. Each arm will have 15 participants (plus an additional 15 partners recruited by the intervention-arm participants). The intervention arm will receive the modified DII intervention whereas the control arm will receive general health education.

Target Population and Intervention Participants

This non-randomized, two-arm trial will enroll and complete measurements on 30 individuals (n=30 control and n=30 intervention). Participants will be recruited from the broader Columbia, SC community, including African-American churches which we have worked with on several research studies conducted through the University of South Carolina to date. We will recruit participants from the community via fliers and listserv messages. The trial will be based at the main offices of the University of South Carolina's Cancer Prevention and Control Program in Columbia. Potential participants will be provided with a study information sheet which outlines inclusion and exclusion criteria (see below) and provides details about the study. If the individual is interested in participating, then they will complete a study contact sheet, which will be given to study staff at Connecting Health Innovations (CHI). Given we will be recruiting from African-American churches, as well as the general community, we expect to enroll mainly African Americans. However, recruitment will not be restricted on race or ethnicity.

Inclusion Criteria:

- Be ≥18 years of age
- Have no serious, unstable co-morbidity that would make participation in a diet and PA intervention difficult or risky
- Be willing and able to participate fully in the study for a period of three months
- Have Columbia, SC area residence and be able to travel to and from the clinic and intervention sites
- Have access to the Internet and a valid email address
- Not be currently enrolled in a weight loss study or actively taking weight loss medications.
- Have a body mass index (BMI, kg/m^2) between 25.0-49.9.

Exclusion Criteria:

- Serious, unstable co-morbidity that would make participation in a diet and physical activity intervention difficult or risky including renal disease retinopathy, peripheral vascular disease or neuropathy;
- Any medications that are known to influence CRP levels such as chronic steroid (e.g., prednisone) use;
- Diagnoses of congestive heart failure, chronic renal failure, chronic liver disease (including alcoholic cirrhosis), cancer within the past year (except for non-melanoma skin cancer);
- Actively receiving cancer treatment;
- Have had any major surgeries in the past 3 months;
- Poor performance status according to the World Health Organization definitions (i.e., Level 3: symptomatic, >50% in bed, capable of only limited self-care; or Level 4: bedbound); or
- Life expectancy <3 years, on hospice, nursing home or other institutionalized care.

Recent attention to behavioral interventions has pointed out limitations in traditional randomization schemes among these types of randomized controlled trials (e.g., inability to truly blind participants, lack of decision making by participants).[20] Other methods of allocation, which take into account perceived ability to comply with intervention requirements, are much more appropriate for this kind of intervention. Therefore, we propose that only individuals who are motivated to make a healthy diet change will receive the intervention.

Recruitment and Consent Procedures

Fliers will be distributed at a couple of African-American churches which have participated in previous Cancer Prevention and Control Program (CPCP) studies, as well as general community locations, including but not limited to USC. Additionally, recruitment efforts will utilize target listserv messages. Once a potential participant is identified, he or she will be provided with a study information sheet which outlines inclusion and exclusion criteria (see below) and provides details about the study. If the patient is interested in participating, then they will complete a study contact sheet, which will be given to researchers at CHI. After an individual has been identified and completed a contact form, CHI staff will contact the patient and conduct a brief phone screener with them. If the individual qualifies, they will be asked to schedule a group orientation meeting time that is convenient for them. Numerous studies, including those conducted by this study's staff, have indicated greater commitment and motivation to participate in health behavior interventions when undergoing that intervention with a family member or close friend. Therefore, intervention participants will be asked to invite one person to the intervention classes.

At this group orientation, participants will learn more about the study and what will be involved. They will be given consent forms to complete. Once consented, they will be instructed on completing the baseline questionnaires and the 24-hour Dietary Recall (ASA24™). Participants also will be instructed on using the Actigraph GT3X-BT for 7 days (more detail is under Participant Expectations) to measure physical activity and sleep. After this meeting, participants will have two weeks before the start of the study. During that time, they will complete the 7 days of wearing the Actigraph and will complete four days of dietary reporting using the 24-hour Dietary Recall (ASA24™).[21] In addition, the participant will visit the CPCP where they will have their height and weight and blood pressure assessed, and laboratory blood work done (more detail is under Participant Expectations). Once visits are completed and at least 7 days have passed, the study coordinator will collect the Actigraph from the participants. Participants selected as controls will complete all of the same measures the primary participants will do but will not receive the intervention and will not need to attend the orientation session.

After all baseline measures are completed, participants will start the weekly group sessions. After at least one class has been completed, the study nutritionist will provide participants with an individualized counseling session to provide individualized guidance on study-related dietary and exercise requirements.

Participation Expectations (Intervention)

One groups of 15 individuals, plus their invited partners, will meet weekly for 12 weeks to cook, and engage in exercise and stress-reduction activities. This 3-month intervention will involve participants' convening weekly for 12 consecutive weeks. Participants will be expected to attend baseline and post-intervention (i.e., 12-weeks) clinic sessions. Intervention classes will meet on the same night at the same time for each of the weekly classes. Participants will have access to online material through our IMAGINE Healthy Online Portal

and will be given take-home activities, referred to as IMAGINE actions. These homework assignments will focus on goal setting for nutrition, physical activity, and stress reduction. The dietary component of the classes will focus on increasing consumption of foods shown to decrease inflammation and limit consumption of foods that increase inflammation. A specific focus will be spent on watermelon. A total of 84 watermelon or watermelon components (e.g., seeds) are being developed as a focal point for this intervention. Participants will be expected to take part in hands-on cooking classes. Participants will be encouraged to obtain at least 30 minutes of moderate intensity physical activity (equivalent to brisk walking) on at least five days of the week and to engage in at least two sessions of strength training each week. To ensure safety, participants will be encouraged to progress very gradually in increasing the intensity and duration of physical activity and only do so within their abilities. The Actigraph has been used extensively with respect to characterizing physical activity.[22-28]. Additionally, sleep characteristics can be obtained when participants wear the Actigraph during sleep, which will be a requirement of this study.[29] Given the limited budget of this mechanism, the Actigraph will need to be provide in two waves. The first wave (about 8 of the intervention participants) will receive the Actigraphs at orientation. The remaining intervention participants will receive them at the baseline clinic. All intervention participants will have completed wearing the Actigraph by the first intervention class. Blood samples will be obtained from participants at baseline and after the 12-week intervention period. Additionally, a series of questionnaires also will be obtained at the two time points, as well as anthropometric measurements including a Bioelectric Impedance Analysis (BIA) scale which is very similar to a standard weight scale. Blood pressure also will be obtained. Participants will be expected to complete 4 24-hour dietary self-reports at each time point. This will be accomplished using the ASA24. All questionnaire data will be developed in Qualtrics, an online survey service. Therefore, all participants are expected to have access to the internet to complete all questionnaires. Additionally, the ASA24 must be completed online.

Participant Expectations (Controls)
Control participants will be selected from the same or similar sources as the intervention participants. The data collection will be the same between the intervention and control participants. The only difference is that the control participants will not be attending any intervention classes. Instead, this group will receive weekly health information. This information will be provided through email or mail, which can include weekly newsletters, healthy recipes that are not focused on reducing the DII scores, links to online content, and health-related event announcements in and around Columbia, SC. Any participants in the standard of care arm who do not use email regularly may request for items to be mailed to their mailing address.

Sources of Materials
Data collection points will be at baseline and after week 12 of participant involvement. Baseline measurements will occur after the participant has been screened for eligibility and has signed the informed consent form as close to the first intervention session as possible. Consistent measurements will occur for all participants.

At each measurement point, the following sources of materials will be collected:

- Informed consent form (at orientation session only; i.e., before any active involvement in the study)
- Blood pressure reading
- Anthropometric measurements, including height, weight, hip and waist circumference, and body fat percent using the BIA machine
- Questionnaires via online Qualtrics
- Diet (i.e. three 24-hour recalls) and physical activity logs indicating activities when the Actigraph armband is not worn
- Actigraph monitor
- Blood samples for CRP, HbA1c, insulin, and lipids (three eight-milliliter tubes will be collected).

Project staff will record the blood pressure of intervention participants using a standard blood pressure cuff after the participant has been resting for 5 minutes. Three readings will be taken with the second and third readings recorded. Staff also will measure body weight and height using an electronic scale and stadiometer, respectively. Hip and waist circumference will be measured using a Gulick™ measuring tape. Body composition will be obtained through BIA performed using the Tanita TBF-300WA Body Composition Analyzer (Arlington Heights, Illinois). Physical activity logs will be used to track participation in physical activity when the Actigraph is not worn. Participants height, weight, age, gender, smoking status, and handedness will be used to calibrate the Actigraph monitors. These monitors will be worn at all times and will only be taken off for

bathing/showers or water activities. Participants will be provided the monitors for 7-10 days to ensure at least 4 days of complete data. Complete data will be at least 23 reported hours of armband wear time or wear time plus information provided in the physical activity logs (e.g., supplementing wear time with time spent swimming). Blood samples will be collected by trained phlebotomists for characterization of the biomarkers identified above. Online questionnaires will be provided to all participants to complete at each data collection point. Project staff will assist with completion as necessary. Questionnaires will include items to assess sociodemographic characteristics, health behaviors, social desirability and approval, self-reported physical activity, stress, depression, and confidence in the ability to make lifestyle changes. Self-report 24-hour dietary recalls will be used to obtain specific information on meals during the previous 24 hours using the ASA24. Lastly, participants will be provided with an online pass to our IMAGINE Healthy Online Portal to provide support and information in relationship to the weekly classes. Access to this material typically requires a fee. Therefore, the intervention participants will gain access through the study, but the controls will not be able to access this material. This limits the likelihood of "exposure contamination" by the controls.

Participants, but not their invited partners, will be provided with a $20 incentive for baseline assessment measures and $20 incentive the 3-month measures. Control participants will receive the same incentives.

Potential Risks

Potential risks include the possibility that some questions included in the questionnaire may make some respondents feel uncomfortable (for example, inquiries about their diet, physical activity, smoking and alcohol habits). However, respondents may refuse to answer any question. The phlebotomy component carries a slight risk associated with venipuncture (redness, swelling, etc.). Phlebotomy will be undertaken by an experienced phlebotomist trained to minimize any risk associated with this portion of the study. All blood draws will take place at USC's CPCP by a trained phlebotomist. There is virtually no risk to the subjects from the lab assays, which are routinely used in clinical practice. Participants may experience discomfort, irritation, or in rare cases, a skin reaction to the Actigraph (worn for 7 days 3 times during the study). However, it is very similar to a wrist watch and any reactions would be rare. We will discuss ways to limit irritation and instruct participants about discontinuing use if an allergic reaction occurs. The BIA machine carries virtually no risk. It is essentially the same as standing on a typical weight scale. However, in between each participant, the scale will be cleaned as it requires the participant to be bare-footed. The intervention itself is very unlikely to cause any serious harms. From our experience, we recognize that a healthy diet intervention will only aid in improving the health of our participants. We will encourage physical activity and provide education on physical activities. However, most of the physical activity will be performed outside of the study environment. Our experience has been that most subjects in our intervention studies in Columbia, South Carolina are pleased to be able to participate in health research.

Statistical Analyses

Sample characteristics were described by intervention condition using frequencies and means. Differences between groups at baseline were derived with Fisher's exact test for categorical covariates given the small sample sizes. For continuous covariates, t-tests or Wilcoxon rank sums test, depending on normality, were used. Differences in daily average watermelon consumption were tested using t-tests.
Outcomes included CRP, HbA1c percent, glucose, BMI, body fat percent, and body weight. Given the small sample size, it was not possible to conduct a confounder selection process. However, all models were adjusted for age. Using general linear models with repeated measurements with a compound symmetry covariance structure, least-square means of the outcomes by intervention status were obtained. Next, an interaction term was added to investigate the intervention-by-time effect. This process was conducted for each outcome.

The IMAGINE-Watermelon program was designed to lower dietary inflammatory potential as measured by the E-DII. In theory, the intervention arm should have lowered their E-DII score (i.e., become more anti-inflammatory) and the control arm should have stayed roughly the same. Adherence to the intervention assignment was examined based on change in the E-DII score. A *post-hoc* analysis was designed to examine whether a change to a more anti-inflammatory dietary pattern led to a change in inflammation regardless of intervention status. Participants were dichotomized at the median based on the change in their E-DII score from baseline to post-intervention. Those below the median had reductions in their E-DII scores (i.e., became more anti-inflammatory) and those above the median became more pro-inflammatory. For this *post-hoc* linear regression analysis, the independent variable was the change in the E-DII scores analyzed as both continuous

and dichotomized at the median. Multiple least square regression was then performed to obtain least square means of the dependent variables by E-DII change adjusted for age. Additionally, the change in E-DII score was examined as a continuous measure where beta coefficients for a one-unit change in the E-DII score were reported. A one-unit increase in the change in E-DII score indicates a worsening of the E-DII score.

Protection against Potential Risks
All procedures used for data collection adhere to high standards of clinical or research practice or both. We have extensive experience with all procedures undertaken under this project. Confidentiality will be maintained by using the randomly assigned study number for each subject. Identifying information will be kept in secure locations (including password-protected computer files and locked file cabinets) with restricted access. All study staff members will have been trained using the CITI® protocol to ensure protection of patient privacy, and will be informed prior to the commencement of the study about the necessity for keeping all data confidential. Laboratory personnel will be blinded to the specific identity of the subjects, and will not be able to link any identifying information to the lab results. Analyses and publication of study results will concern group data only; it will not be possible to link any such results to information about any particular study participant or to deduce participant identity from published data. We note potential alternative to harms in the previous section for each of the data collection sources.

Potential Benefits of the Proposed Research to the Participants and Others
This study has the potential to confer benefit to the patient by reducing her or his chronic, systemic inflammation. Besides any direct health benefits conferred, the participant will receive a report of all data collected. The information we collect will be used to form the foundation of a future DII ‘App’ and counseling protocol and, as such therefore could inform approaches to prevent inflammation-related health problems in the U.S. (and elsewhere in the world).

Importance of Knowledge to be Gained
The knowledge generated by the research will provide an understanding of the effect of the developed program in improving diet and reducing inflammation from dietary sources. Additionally, this will help determine the effectiveness of focusing on specific foods, in this case watermelon, in our IMAGINE program.

Inclusion of Women and Minorities
All subjects will be U.S. citizens who are suspected of suffering from diet-associated chronic, systemic inflammation. The condition under study, chronic systemic inflammation is rampant in the US population; however, it is somewhat more prevalent among African Americans than in European Americans. Because we are recruiting from African-American churches and the general community of South Carolina, we expect that at least 75% of our population will be African American. Chronic, systemic inflammation afflicts both sexes and dietary quality, and therefore associated DII, will be well represented in both of the sexes. Therefore, we will attempt to recruit women and men in equal numbers. Men and women will be recruited and followed using the same procedures; therefore we anticipate that women and men will be recruited in the same numbers.

Inclusion of Children
Although children can suffer from diseases related to chronic inflammation these conditions and the underlying risk factors are much more common in adults. Therefore, children will not be enrolled in this research.

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