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Effects of fermented vegetables on markers of inflammation and composition of the intestinal microflora in overweight and obese women

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Abstract

Overweight and obese individuals seem to have an altered profile of the gut microflora compared to normal weight individuals. This proposal will examine whether daily consumption of fermented vegetables for 6 weeks can impact the gut microflora and markers of inflammation of overweight and obese women.

Problem to be studied and its significance

Despite significant research efforts from the public and private sectors to reduce the burden of obesity, it continues to affect individuals worldwide [1]. In the United States, the prevalence of obesity among U.S. adults during 2011-2014 was 36.5% [2]. It is worthy to note, however, that during this period, the prevalence of obesity in women aged 20 to 59 years was significantly higher than in men (38.3% in women versus 34.3% in men), and if race is taken into consideration, non-Hispanic black women have the highest prevalence of obesity (56.9%) followed by Hispanic (45.7%), and non-Hispanic white women (35.5%) [2].

Over the last decade there has been an increasing interest in the relationship between the intestinal microflora and obesity as several research studies have shown an association between obesity and changes in the composition of the intestinal microflora [3-6]. For example, *Bifidobacteria*, which are regarded as probiotic bacteria that confer health benefits to the host, are found to be decreased in fecal samples of overweight and obese subjects compared to lean subjects [7]. In addition, obese subjects seem to have a lower ratio of *Firmicutes* to *Bacteroidetes*, which are some of the most abundant bacterial phyla present in the human gut [8]. Some studies suggest that the altered intestinal microflora profile of obese individuals may lead to an increase in production of lipopolysaccharide (LPS) endotoxin, which is derived from the cell wall of gram-negative bacteria in the gut (such as the *Bacteroidetes* mentioned earlier) and induces an inflammatory response by the body [9]. This may be one reason why overweight and obese individuals often present elevated levels of inflammatory markers in the blood, including LPS [10] and other more established markers of inflammation such as C-reactive protein (CRP) [11].

The composition of the intestinal bacteria is influenced by several factors, including host genotype, age, sex, antibiotic/medication use, disease, and diet. Changes in the intake of specific food components may lead not only to changes in the gut bacteria but may also lead to changes in gene expression of different tissues in the host, which may be directly related to more or less fat storage [12]. For example, a cross-over study looking at the effects of consumption of 4 weeks of fermented kimchi and 4 weeks of fresh kimchi in obese Korean subjects (7 male and 15 female) found significant decreases in body weight, and body fat after consumption of fermented and fresh kimchi. In addition, decreases in blood pressure, percent body fat, fasting glucose, and total cholesterol were significantly greater during the fermented kimchi phase compared with the fresh kimchi phase [13]. In a parallel arm study conducted with overweight and obese Korean women, consumption of both fermented and fresh kimchi resulted in a decrease in the *Firmicutes* to *Bacteroidetes* ratio and in the relative abundance of *Bifidobacteria,* even though there were no significant differences between groups in body weight or inflammatory markers [14]. The findings from these Korean studies suggest that fresh and fermented kimchi may have different effects on intestinal bacteria and metabolism of the host. Unfortunately, very few studies examining the effects of fermented foods on metabolic parameters have been conducted in the U.S., likely due to the low consumption of fermented vegetables by the population. The present study was designed to investigate the effects of fermented vegetables on the composition of the gut microflora and other biological and clinical parameters associated with obesity and other chronic diseases.

Study Aims

Aim 1: To examine the effect of daily consumption of fermented vegetables compared with non-fermented vegetables for 6 weeks on markers of metabolic syndrome and inflammation of overweight and obese women.

Hypothesis 1: Consumption of fermented vegetables will improve markers of metabolic syndrome (blood pressure, insulin, adiposity) and inflammation, such as C-reactive protein (CRP), and lipopolysaccharide (LPS) in overweight and obese women.

Aim 2: To examine the effect of daily consumption of fermented vegetables compared with non-fermented vegetables for 6 weeks on the profile of the gut microflora.

Hypothesis 2: Consumption of fermented vegetables will lead to a shift in microbial communities towards an increase in communities regarded as probiotics such as Bifidobacteria and Lactobacilli.

Significance

Obesity continues to be a major public health burden and its consequences have a significant impact on disease risk and quality of life, particularly in women who are more affected by this problem than men. Several weight loss interventions have been tested in the past 30 years, but it does not seem that a great impact in obesity treatment has been made so far. This is partially due to the fact that weight loss trials have a transient positive effect, which gets reversed as soon as the intervention stops. Due to these challenges faced by traditional weight loss trials, there is a need to look for alternative approaches to reduce the burden of obesity, particularly in women. Although it is now well recognized that there is a strong relationship between the gut bacteria and obesity, there is a lack of feeding studies aimed at altering the composition of the gut bacteria via consumption of fermented foods. In addition, most of the studies examining the effects of fermented foods on health and disease parameters have been conducted in Asian countries, where the consumption of fermented foods like kimchi and soy products is high compared with Western countries. This proposal addresses the gap in the research with fermented foods that exists in the U.S. by examining the effects of consumption of fermented vegetables on several markers associated with obesity in women.

Methodology and analysis

Methodology

Study design: This will be a parallel arm study where 39 women will be randomized into one of three treatment groups: a fermented vegetable group (n=13), a non-fermented vegetable group (n=13), and a control group (n=13). The duration of the study will be 6 weeks and women in the vegetables groups will be asked to consume 1 cup of vegetables (140 g) per day for the entire duration of the study as well as follow their usual diet. Women randomized into the control group will be asked to follow their usual diet. Biological samples including feces and blood samples will be collected at baseline, before randomization and at the end of the 6-week intervention.

Study participants: Thirty-nine overweight and obese women aged 20-59 years will be recruited for this study. The table below shows inclusion and exclusion criteria for the study:

INCLUSION CRITERIA	EXCLUSION CRITERIA
BMI: 26-40 kg/m^2	BMI < 26 kg/m^2
Non-smoker	Smoker

No previous diagnosis of cancer	Taking medications that affect appetite or body weight
No thyroid disease	Uncontrolled Hypertension
No diabetes	Diabetes
Willing to consume 1 cup of vegetables daily for 6 weeks	Not willing to consume 1 cup of vegetables daily for 6 weeks
No use of psychotic or depression medication	Willing to show up at two appointments
No medication to lose weight	Following a fad diet
Not on a weight loss diet	Using antibiotics frequently
No use of antibiotics over the past 3 months	Diagnosed with autoimmune disease, like psoriasis, rheumatoid arthritis, thyroid disease, colitis
No consumption of fermented vegetables on a regular basis	Regular consumption of fermented vegetables
No history of autoimmune disease, including gastrointestinal disease	

Study procedures: Participants will be recruited by posting flyers at UNF Women's Center and in Jacksonville Women's clinics, as well as by newspaper advertisements. Interested participants will be asked to contact the study via email sent to an email account that will be created for the study. Upon receiving emails from interested participants, study staff will conduct a screening interview to ensure eligibility for the study. If women are found to be eligible after the initial screening interview, a screening clinic visit for verification of eligibility status will be scheduled. At the screening clinic visit all the study procedures will be explained in detail, and participants will be asked to sign the consent form if they agree to participate. Blood and stool samples will be collected and once all eligibility criteria are confirmed, participants will be randomized into one of the three groups and arrangements will be made for weekly pick up/delivery of vegetables to those in the vegetables groups.

<u>Vegetables preparation:</u> Fermented vegetables for the entire study will be provided by a local producer weekly, whereas non-fermented vegetables samples will be obtained at a local grocery store. The process of vegetable fermentation involves *Lactobacilli* and *Bifidobacteria* that are naturally found on the leaves of cabbage, carrots and on cucumbers. These bacteria are able to convert the carbohydrates present in these vegetables into lactic acid under anaerobic conditions. The lactic acid is responsible for the acidic flavor of the fermented vegetables. The non-fermented vegetables will serve as a positive control for the fermented vegetable group, because it will have a similar flavor but no live microorganisms, mainly because store-bought pickled vegetables and sauerkraut are made by a different process that uses acetic acid, instead of live bacteria. Vegetable samples will be portioned by study staff using food safety precautions and delivered to participants weekly.

<u>Biological sample collection:</u> Blood samples will be collected in red top tubes to obtain serum for determination of insulin, CRP, and LPS. CRP will be measured using a commercial ELISA kit (Cat#DCRP00, R&D Systems, Minneapolis, MN). LPS will be measured using Pierce LAL chromogenic endotoxin quantitation kit (Cat#88282, ThermoFisher Scientific, Waltham, MA). All analyses will be conducted in Dr. Arikawa's laboratory, which is already set up for these measurements. Stool samples will be collected to analyze the composition of the gut bacteria. Participants will receive a stool collection kit prior to the initial screening visit and they will be asked to bring the stool sample to the screening visit.

Survey data collection: Participants will be asked to fill out several surveys to assess food intake, demographics, and prescription medication intake. The diet history questionnaire, which is a 120-item food frequency questionnaire designed by the National Cancer Institute [15] will be used to assess baseline and follow-up dietary intake of participants. Participants will also receive daily logs to monitor gastrointestinal function (frequency of defecation and consistency of stools) and possible side effects of consumption of fermented vegetables such as bloating, diarrhea, constipation, and headache.

Clinical data collection: Height will be measured in centimeters using a Detecto 439 Eye Level Beam Physician Scale 400lb x 4oz with Height Rod. Weight and %body fat will be obtained via Bioimpedance (Tanita BC-532). Sagittal abdominal diameter (a measure of visceral obesity) will be assessed using a Holtain-Kahn caliper. Blood pressure will be measured using a Digital blood pressure monitor (Omron HEM907XL).

Compliance: To assess compliance in the vegetables groups, participants will be asked to fill out logs where they will check whether they consumed the vegetables for the day or not. Participants will also be asked not to discard any vegetables that have not been consumed. These containers will be picked up weekly at the time participants receive their following week's supply. This will allow us to quantify the amount of vegetables that is truly consumed by the participants every week.

Microflora analysis: Stool samples will be stored at -70C until DNA extraction. DNA will be extracted using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) and samples will be stored until analysis of microflora. Analysis of microflora in stool samples and vegetables samples will be conducted in the laboratory of Dr. Ryan Fink at St. Cloud State University. This type of analysis is currently not being performed at UNF, therefore, Dr. Arikawa will be working on a contract to have the samples measured at St. Cloud State University. Drs. Arikawa and Fink have had a working relationship since 2012 when they worked on a study related to fermented wheat bran and its effects on the composition of the gut microflora at the University of Minnesota.

Analysis

Treatment groups will be compared at both times points using analysis of variance. Changes from baseline within treatment groups in clinical and laboratory measures will be compared by paired *t* tests. Pearson's correlation coefficients will also be calculated between clinical and laboratory parameters and alterations in the intestinal microflora.

Plan for dissemination of results

The findings from this project will be disseminated in three different ways: via open access peer-reviewed manuscripts, poster presentations at scientific meetings, and via UNF newspaper. It is also expected that this project will originate pilot data to apply for external funding from institutions such as the Obesity Society and the American Heart Association.

Timeline

Project activities	Year 1				Year 2			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
IRB application and approval								
Recruitment and enrollment of participants								
Intervention/data collection								

Sample preparation for analysis and shipping								
Intestinal microflora analysis at St. Could State University								
Statistical analysis								
Manuscript preparation and dissemination								

Project budget ($14,975.65)

Undergraduate research assistant **($1,431.75)**

I am requesting funds to hire an undergraduate student for 7 hours per week to help with recruitment and the intervention for 19 weeks at $10/hr. ($10*19*8 = $1330 + 7.65% = $1,431.75).

Graduate student (nurse practitioner) ***($280)***

I would like to hire nurse practitioner students for the blood draws. I estimate that each blood draw will take 15 minutes, which adds up to 10 hours for 39 participants before and 10 hours after the intervention for a total of 20 hours. ($13/h for 20 hours = $260 + 7.65% = $280)

Laboratory supplies

Assay kits for assessment of blood markers ***($2,362)***

CRP: 1 kit for 36 samples at $479, need 2 kits = $958**,** Insulin: 1 kit for 36 samples at $449, need 2 kits = $898**,** LPS: 1 kit for 36 samples at $253, need 2 kits - $506

Blood and stool collection supplies ***($433)***

Needles, tubes, tourniquets: $150, Tubes to store serum in ultracold freezer: $159, Stool collections cups and hats: $108, Tongue depressors: $16

Microflora analysis ***($8,500)***

DNA extraction of stool samples: $900

Sequencing for identification of microflora $1,500 per 17 samples, we will have a total of 84 samples: $7,500

Shipping (overnight) of stool samples to Minnesota: $100

Vegetables ***($900)***

Commercial sauerkraut for non-fermented vegetables group ($44.30 per 384 oz – need 223 oz per person for the entire study, 223 x 13 people= 2899 oz = 8 cases): $355

Fermented vegetables ($29 per 256 oz – need 223 oz per person for the entire study): $377

Storage containers: (need 1 cup containers: 42 days x 39 participants = 1638 containers, $24 per case of 240, need 7 cases): $168

Participant compensation **($780)**

$20 dollars for completing the study, $5 for completing baseline measures and $15 for completing the 6 weeks. Compensation will be pro-rated if participants drop out before 6 weeks.

Car mileage **($288.90)**

Vegetables samples will be delivered to participants weekly for 6 weeks. Assuming that we will drive 15 miles for every 5 participants per week = 15 miles x 6 (28 participants in the vegetables groups divided by 5 = 6) x 6 weeks = 540 miles x $0.535 = $288.90

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