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Feasibility outcomes of a pilot randomized clinical trial to increase cruciferous and green leafy vegetable intake in post-treatment head and neck cancer survivors

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Abstract

Background: Higher intakes of cruciferous vegetables (CV) and green leafy vegetables (GLV) in observational studies are associated with improvements in survival and cancer-related biomarkers in patients diagnosed with head and neck cancer (HNC). These results have yet to be corroborated in a randomized clinical trial (RCT).

Objective: Determine the feasibility of implementing a 12-week RCT to increase CV and GLV intake in post-treatment HNC survivors.

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Author contributions:

AA, WC, SS, JL, WDW, LR designed the study. SC, AF, AD, AA conducted the study. JE and MB performed carotenoid analysis and LM performed DNA methylation analysis. YC and KD assisted with statistical methods. SC and AA wrote the first draft of the manuscript with contributions from all authors. All authors reviewed and commented on subsequent drafts of the manuscript.

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Conflict of interest disclosure:

The authors declare that they have no competing interests.

Design and participants: This was a two-arm, RCT conducted among 24 post-treatment HNC survivors. Survivors were recruited from a southeastern, National Cancer Institute-designated Comprehensive Cancer Center between 01/2015 – 09/2016.

Intervention: (1) experimental group (n=12) receiving weekly 15–30 minute telephone dietary counseling from a RDN stressing 2.5 cups/week CV and 3.5 cups/week GLV or (2) an attention control group (n=12) receiving weekly 15–30 minute telephone dietary counseling from a RDN focusing on general healthy eating for cancer survivors. Participants completed a baseline survey, three 24-hour dietary recalls, phlebotomy, and anthropometric measures prior to randomization and at the end of the 12-week study period. The experimental group also completed weekly vegetable record recalls.

Main outcome measures: Feasibility-recruitment, retention, adherence, and safety. Secondary outcomes included inflammatory markers and carotenoids.

Statistical analyses performed: Descriptive statistics were generated for demographic, epidemiologic, and clinical variables as well as the primary feasibility outcomes. Between- and within-group comparisons of mean serum cytokine and carotenoid levels were performed using appropriate statistical tests depending on their respective distributions for the purpose of generating preliminary effect sizes.

Results: 350 incident HNC cases were screened for eligibility. N=98 were eligible for study participation. Reasons for ineligibility and exclusion included: “deceased” (n=93), “wrong/inactive numbers/unable to reach/lost to follow-up” (n=93), not meeting inclusion criteria (n=39), and “too ill to participate” (n=27). Of the n=98 eligible HNC cases, n=24 agreed to participate, enrollment rate 25%. The most common reason for non-participation was “distance” (n=48), as participants were asked to report for two on-site assignments. The retention rate was 96%. Mean intervention adherence rates for weekly goals were 67% CV, 74% GLV, and 71% overall. Completion rates of weekly counseling calls was 90%. The experimental group reported an overall mean increase of 5.5 GLV and 3.5 cups of CV per week from baseline intake, respectively. No significant between- or within arm differences were observed for inflammatory markers or carotenoids.

Conclusion: A post-treatment intervention aimed at increasing CV and GLV intake in HNC survivors is feasible. A larger RCT is needed to assess the efficacy of this intervention on disease outcomes.

Keywords

head and neck cancer; dietary intervention; cruciferous vegetables; green leafy vegetables; randomized clinical trial

Introduction:

Nutritional interventions have the potential to improve dietary intake in cancer patients who are at risk for malnutrition^{1, 2} and promote improved quality of life (QOL) and disease prognosis regardless of tumor type^{3, 4}. However, the majority of research studies testing diet interventions to date have focused their attention on breast, colon, and prostate cancers^{1, 5, 6}. This has left other cancer survivor populations considerably understudied, including head and neck cancer (HNC) survivors. HNC survivors experience exceptionally high rates of

physical and psychological burden into survivorship, including fatigue, and reduced physical functioning and overall QOL^{7–13}. To date only two randomized controlled trials (RCTs) aimed at increasing fruit and vegetable intake in HNC survivors have been conducted, and both targeted early-stage HNC patients at risk for second primary cancers rather than late-stage HNC patients, which make up the majority of the HNC population^{14, 15}.

Observational studies of HNC patients have suggested that a dietary pattern characterized by high consumption of cruciferous vegetables (CV) (e.g. broccoli, cabbage, cauliflower) and green leafy vegetables (GLV) (e.g. spinach, kale), fruit, poultry, fish and whole grains is associated with better clinical and supportive care outcomes. These outcomes include more favorable DNA methylation profiles¹⁶ and reduced levels of pro-inflammatory cytokines¹⁷, as well as reduced symptom burden¹⁸, recurrence and mortality rates¹⁹. These studies were the first to provide evidence of such associations in HNC, and no study, to date, has tested if the associations persist in a randomized controlled trial (RCT).

Up to 47% of HNC patients do not adhere to nutritional counseling^{20, 21}. High symptom burden, (e.g., xerostomia, dysphagia, and taste alterations) persist among many HNC patients post-treatment and possibly hinders the ability or desire to eat²². For many patients, a major component of QOL is the ability to enjoy eating²³. Therefore, studies assessing the feasibility of a dietary intervention that is attentive to the unique eating challenges of this population, while aiming to increase intake of foods that may have beneficial effects on survival outcomes are necessary. As such, a 12-week, two-arm, pilot RCT was implemented to assess the feasibility of a post-treatment intervention to increase CV and GLV intake in HNC survivors. The primary study aim was to determine feasibility outcomes, including recruitment, retention, intervention adherence, and safety. Secondary outcomes included potential changes in circulating levels of cancer-related biomarkers (pro-inflammatory cytokines, carotenoids, and patterns of cell-free DNA methylation).

Methods:

Design and Setting

This was a two-arm, RCT conducted among post-treatment HNC survivors designed to test the feasibility of a dietary intervention to increase cruciferous (CV) and green leafy vegetable (GLV) intake. HNC survivors were recruited from a southeastern, National Cancer Institute-designated Comprehensive Cancer Center. Twenty-four patients treated for HNC at the cancer center who were six months post-treatment, were enrolled and randomized to either an experimental or attention control group. Outcomes were assessed at baseline and 12-weeks. The protocol was approved by the Institutional Review Boards of the University of Alabama at Birmingham and the University of Illinois at Urbana-Champaign and all participants provided written informed consent. This clinical trial was registered under [ClinicalTrials.gov](https://clinicaltrials.gov) study identifier NCT03013699.

Participants and Procedures

Screening and recruitment occurred between January 2015 – September 2016. Names and contact information of potentially eligible participants were obtained through the

institutional cancer registry. After confirming eligibility via the Electronic Medical Record (EMR), potential participants were contacted via telephone and introduced to the study. Inclusion criteria included: (1) diagnosed with Stage I - IV oral, hypopharyngeal, nasopharyngeal, oropharyngeal, or laryngeal cancer; (2) age 19+; (3) at least 6 months – 2 years post-treatment; (4) able to consume foods orally; (5) no evidence of disease; (6) English-speaking. Exclusion criteria included: (1) dementia or organic brain syndrome; (2) active schizophrenia; (3) another diagnosis of cancer in the past five years (not including skin or cervical cancer in situ). The EMR and online obituaries were searched to prevent calling individuals who were deceased. Participants who indicated during the recruitment telephone call that they were not interested in the study were thanked for their time and asked to volunteer their reason for disinterest; this information was de-identified and recorded. If potential participants were interested in the study, the consent form was verbally reviewed and a copy was sent to the participant via e-mail or first class mail. The participant was then scheduled for a baseline study visit at the institutional Clinical Research Unit. At the baseline study visit, the informed consent was re-reviewed with the participant, and the participant was given the opportunity to ask questions. Once all concerns were satisfied, formal written consent was obtained.

All study participants were asked to report for two on-site assessment visits (baseline and 12-week follow-up) at the institutional Clinical Research Unit. The baseline assessment included: (1) a questionnaire collecting data on demographics, comorbidities, smoking and alcohol use; (2) anthropometric measures (weight, height); (3) phlebotomy; and (4) a 24-hour dietary recall. Participants were asked to bring two detailed dietary records to each of the study visits which were reviewed by the RDN. All measures completed at baseline were repeated at the 12-week assessment, with the exception of time invariant variables, e.g., demographics and height.

To minimize participant burden related to travel, efforts by study staff were made to schedule the baseline and 12-week study assessments at the time of participants' usual cancer surveillance appointments with their oncologist.

Randomization

Participants were randomized after informed consent was obtained and baseline assessment was completed. Randomization assignments were computer generated and kept in sealed envelopes to prevent bias in group allocation by study personnel. Participants were randomized to one of the two study arms: (1) the experimental arm that received weekly CV and GLV counseling or (2) the attention control arm.

Dietary Intervention

Attention Control Arm—Half of the participants (n=12) were randomized to an attention control arm and received educational materials on healthy eating for cancer survivors that have been developed by the American Institute for Cancer Research (brochure #369–0613) and widely used by RDNs working in oncology nutrition²⁴. Participants in this group received weekly individualized dietary counseling focusing on healthy eating for cancer survivors and addressing chronic side effects of treatment that might hinder the ability

and/or desire to eat. Counseling sessions occurred weekly during the 12-week study period and lasted 15–30 minutes each, depending on the participant's needs. As many of the potential study participants did not live within feasible weekly driving distance to the cancer center, dietary counseling sessions that took place between the baseline and follow-up study visits were conducted via telephone.

Experimental Arm—The other half of the participants (n=12) were randomized to receive the same weekly, one-on-one dietary counseling with educational materials on healthy eating for cancer survivors with an additional focus on meeting weekly goals for CV and GLV intake. At the baseline study visit and after randomization, participants in the experimental group received an educational folder that included pamphlets with colorful pictures and descriptions of types of foods classified as CV and GLV as well as recipes for dishes containing these vegetables. The RDN addressed potential disease- and treatment-related eating challenges experienced by each participant that may serve as barriers to intervention adherence (e.g., difficulty swallowing, taste alterations) and helped the participant develop ways of preparing vegetables to make them palatable and easy-to-swallow. Dietary counseling sessions occurred weekly for the 12-week study period and lasted 15–30 minutes each, depending on the educational needs of the participant.

Weekly goals for the experimental group were to consume 2.5 and 3.5 cups of CV and GLV, respectively. Intervention goals were based on the range of servings per day that were reported by HNC patients consuming the highest intakes of these vegetable groups in the University of Michigan Head and Neck Specialized Program of Research Excellence study population (n=542), a longitudinal cohort of newly diagnosed HNC patients in Arthur et al., 2013¹⁹. Intakes of cruciferous and green leafy vegetables were found to be significantly associated with decreased mortality and this association was strongest for participants reporting the highest intakes (Quartile 4; Table 1 – online only)¹⁹. Dietary counseling applied strategies based on the Social Cognitive Theory while focusing on positive behavioral change for improving self-efficacy, skills development, self-monitoring and adherence²⁵. Participants were asked to keep a daily log of their consumption of CV and GLV. No additional measures such as more frequent contacts or different approaches were instituted if a participant was not adherent. However, the RDN discussed perceived barriers to adherence with participants and participants and the RDN worked together to develop ways to overcome them.

Diet Records and 24-Hour Recalls—Dietary intake was assessed for all participants prior to randomization (baseline) and at 12-weeks. Participants were e-mailed or mailed two recording forms and instructed to keep two days of detailed diet records prior to their baseline and 12-week study visits. Participants were educated on how to complete the dietary records to help them record relevant details for all foods and beverages consumed, such as brand name, preparation methods, portion size consumed (weight or volume) and time/place consumed. Upon bringing the food records to their study visits, participants were probed for further detail using the automated multi-pass method by a trained study staff member as needed. A third day of dietary intake was completed at the study visits by conducting a 24-hour food recall interview using the United States Department of

Agriculture (USDA) multiple-pass method^{26, 27}. Diet records and 24-hour recalls were used for measuring dietary intake^{28, 29}. Efforts were made to ensure that the three days of dietary intake collected included two weekdays and one weekend day, and were usual days of intake (e.g., not collected on a holiday).

Food and supplements reported in diet records and 24-hour recalls were entered into and analyzed using the University of Minnesota Nutrition Data System for Research (NDSR) program software version 2015³⁰. The NDSR food and nutrient database contains approximately 18,000 foods, including generic prepared and brand name foods, allowing for an accurate estimate of total energy and nutrient intakes based on reported intake of food and supplements³⁰. Additionally, participants randomized to the experimental group were asked to keep a daily log of their CV and GLV intake as a means of tracking adherence to the intervention. Adherence to weekly goals was assessed using both data collected during the weekly calls and from the pre- and post-24-hour recalls. The three pre-intervention 24-hour recalls were used as the baseline CV and GLV intake measure. The mean and median weekly recalls of weeks two through eleven in addition to the three 24-hour recalls from week twelve were used to determine if an increase or decrease in CV or GLV intake occurred during the intervention period.

Primary Outcome: Feasibility

The study team tracked the following feasibility outcomes weekly throughout the 12-week study period: (1) patients excluded or not agreeing to participate; (2) completion of study assessments and weekly counseling sessions; (3) adherence to vegetable goals (experimental group only); (4) attrition; and (5) adverse events. Participant satisfaction and intervention input were assessed by survey at the 12-week assessment visit.

Secondary Outcomes: Cancer-related Biomarkers

Blood Collection—At the baseline and 12-week study assessment visits, non-fasting whole blood (8.5 mL serum separator tube) were drawn via venipuncture by a trained phlebotomist at the University of Alabama-Birmingham Clinical Research Unit. Participants were asked to avoid smoking, alcohol, and exercise for 24 hours prior to collection primarily because of their acute effects on serum cytokines^{31–34}. Serum samples for cytokines were processed and frozen using standard operating procedures consistent with expert consensus recommendations³⁵ and four 0.5 mL serum aliquots were prepared and stored at -80°C until batch-analysis for cytokines and carotenoids. An additional 4 mL EDTA-treated tube was drawn from study participants at each visit for DNA methylation analysis³⁶.

Serum Cytokines—Serum cytokines were assayed in the University of Alabama-Birmingham Metabolism Core using MSD imager (MesoScale Discovery, Gaithersburg, MD; chemiluminescence technology; multiplex platform). The multiplex platform assayed levels of C-reactive protein (CRP), interferon- γ (IFN- γ), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α).

Serum Carotenoids—Carotenoid analysis was performed in the Carotenoid Analysis Lab at the University of Illinois at Urbana-Champaign. The carotenoid analysis lab takes part in

the NIST Micronutrients Measurement Quality Assurance Program^{37–39}. Analysis was conducted under yellow lights to minimize light damage of carotenoids. Approximately 250 µL serum samples were mixed with an equal volume of ethanol containing 0.1% BHT and were vortexed for 30 s. One mL of hexane was added, vortexed and centrifuged at 2400 rpm at 4 °C for 3 minutes. The hexane extraction step was repeated 2 times, and extracts were combined and dried under argon before being reconstituted in mobile phase for HPLC analysis. The extracts were separated on a reverse-phase C 30 column (4.6 × 150 mm, 3 µm; YMC, Wilmington, NC, USA) maintained at 18 °C. The gradient method used for carotenoid separation was based on the method of Yeum et al.⁴⁰. All analyses were carried out on an Alliance HPLC system (e2695 Separation Module) equipped with 2998 photodiode array detector (Waters, Milford, MA, USA). Individual carotenoids analyzed include lycopene, lutein, zeaxanthin, β-carotene, cryptoxanthin, and lutein.

DNA Methylation—Genomic DNA was extracted from whole blood using the DNA Purification from Buffy Coat protocol from the Gentra Puregene Blood Kit (Qiagen, Hilden Germany). DNA methylation was measured via the HumanMethylation 450 BeadChip Array (Illumina, San Diego, CA, USA). Microarray data analysis was performed in R using the minifi and limma packages. To ensure acceptable sample quality, a detection p-value was calculated for each sample based on the number of failed probes, and only samples with significant p-values were included in the analysis. Next, stratified quantile normalization was performed and M-values were calculated for each probe for each subject⁴¹. Probes were filtered to remove any probe that had a SNP at a CpG site, failed in at least one sample, or was located on a sex chromosome due to the inclusion of both men and women in the study. The probes were annotated with the hg19 genome and the limma package was used to test for pairwise methylation differences between control and experimental groups before and after intervention. A false discovery rate (FDR) p-value < 0.05 was considered significant.

Covariates

Anthropometric measures were conducted in accordance with the Anthropometric Standardization Reference Manual⁴². Current height and weight were collected by trained research staff at each assessment visit, and used to calculate body mass index (BMI; kg/m²). Height was measured to the nearest 0.1 cm (without shoes). Weight was measured on a calibrated platform scale to the nearest 0.1 kg (without shoes and in light clothing with pockets emptied). Demographic characteristics (e.g. age, sex, ethnicity/race), lifestyle behaviors (e.g. smoking/alcohol use), and comorbidities (e.g. heart conditions, diabetes, depression) were self-reported on an epidemiologic health survey similar to those used in previous studies^{19, 43, 44} using the Functional Comorbidity Index, which asks participants to report history of 18 different health conditions⁴⁵. Cancer specific clinical variables including tumor site, cancer stage, and treatment modality were obtained from review of the EMR.

Statistical Analyses

Benchmark goals for feasibility outcomes were set as follows: retain 85% of participants randomized, observe 80% intervention adherence, and 85% outcome assessment completion⁴⁶. Descriptive statistics (means and frequencies) were generated for demographic, epidemiologic, and clinical variables as well as the primary feasibility

outcomes. As this was a pilot/feasibility study there was no anticipation of finding statistically significant differences in inflammatory cytokine levels, serum carotenoids, or DNA methylation between or within groups. However, overall comparisons of mean serum cytokine and carotenoid levels within- and between-groups were performed using appropriate statistical tests depending on their respective distributions for the purpose of generating preliminary effect sizes. For within-group analysis, the sign test was used when the distribution of the difference was not normal and paired t-test was used otherwise. For between-group analysis, the Wilcoxon rank-sum test was performed when either group did not have normal distribution. Two-sample t-tests were performed when both distributions were normal. The sign test was used for analyzing the following carotenoids: β -carotene and xanthophylls in control and cryptoxanthin in both control and experimental groups and for the following cytokines: IFN- γ and IL-1 β and IL-6 in control, CRP and IL-1 β and TNF- α in experimental. The sign test was used for the within group CV analysis in the control group. Paired t-tests were performed on carotenoids: lycopene, lutein, zeaxanthin and total carotenoids in both control and experimental groups, β -carotene and xanthophylls in experimental, and on cytokines: CRP and TNF- α in control, IFN- γ and IL-6 in experimental. Paired t-tests were used for CV analysis in the experimental group, GLV in control and experimental group, and total vegetables in control and experimental groups. For between-group analysis, Wilcoxon rank-sum test were performed on carotenoids: β -carotene, xanthophylls and cryptoxanthin; all cytokine levels were tested using Wilcoxon rank-sum test as well as CV intake. Lycopene, lutein, zeaxanthin and total carotenoid levels were tested using two-sample t-test as well as GLV and total vegetable intake. Both the experimental and control groups were combined into one study population and Spearman correlations were computed between self-reported CV and GLV intake and serum nutrient concentrations at baseline and 12-weeks. Statistical analyses were performed using SAS software, version 9.4 or later⁴⁷.

Results

Study Population

Characteristics of the study population are displayed in Table 2. The sample consisted of 24 HNC survivors living within or around the Birmingham, Alabama metropolitan area and were randomized to either an experimental group (n = 12) or an attention control arm (n = 12). The majority of participants were Non-Hispanic White (83%), male (79%), and had at least some college education (63%). Half of the participants reported a yearly income greater than \$50,000/year and were 3 years or more post-treatment. The average age was 59 ± 8.17 years. Most participants had Stage 3 or 4 disease, however, there was no statistically significant difference in stage between study groups. Two in the control group and five in the experimental group were stage 1 or 2, respectively. Cancers of the larynx and oropharynx comprised the majority of cases. There were no differences between groups in terms of age, sex, time since treatment completion, race/ethnicity, treatment, BMI, smoking status or alcohol consumption. While the sample size was small, 13% were African American and 4% were Pacific Islander. The majority of participants were overweight (mean BMI = 28.1 kg/m²).

Primary Outcome: Feasibility

Accrual—Accrual was met within a 1.5 year time period and required screening 350 incident HNC cases. Of these, n=98 were eligible for study participation and n=252 were ineligible or excluded. Of the 98 eligible HNC cases, n=24 agreed to participate for an enrollment rate of 25% (Figure 1). Reasons for ineligibility were “deceased” (n=93), “not meeting inclusion criteria” (n=39) and “too ill to participate” (n=27). The most common reason for non-participation was “distance” (n=48). Other reasons for non-participation included “not interested” (n=19) and “too busy” (n=5).

Retention and Assessment Completion—Retention exceeded the benchmark of 85% with 23 of the 24 participants (96%) completing the trial. The individual who dropped-out did so because of the time commitment to the study. All retained participants completed all aspects of the assessments.

Adherence—Once enrolled, participants exceeded the previously set benchmark for adherence (80%) as the mean adherence rate for completion of weekly telephone counseling was 90% (Table 3). The experimental group adherence rate to weekly telephone counseling was 86% while the control group was 95%. Mean intervention adherence rates for experimental group self-reported diet record weekly goals were 74% for GLV, 67% for CV, and 71% overall. At baseline, the median intake of CV and GLV for both groups was 0 cups. At 12-weeks, the experimental group reported a median intake of 3 cups for both CV and GLV. The control group reported a median intake of 0 cups of CV while cups of GLV increased to 2 at the follow-up assessment. Additionally, participants in the experimental group reported an overall mean increase of 5.5 GLV and 3.5 cups of CV per week from baseline (pre-intervention) intake, respectively.

Within- and between-group intervention adherence analyses can be found in Table 4. For within-group analysis, the experimental group demonstrated significant mean difference in CV intake before and after the intervention ($p = 0.04$). For between-group analysis, the mean change in CV and GLV intake (i.e., from baseline to 12 weeks) were not significantly different between the control and the experimental groups (Table 3). The between group effect sizes were 0.2 for CV, 0.04 for GLV, and 0.16 overall.

Safety—The risks involved for study participation were minimal; however, safety was monitored and one participant in the attention control arm experienced a transient ischemic attack (TIA) that was deemed to not be study related. No adverse events were observed with the experimental intervention.

Secondary Outcomes: Cancer-related Biomarkers

Serum Cytokines—CRP, IFN- γ , IL-1 β , IL-6, and TNF- α were assessed pre- and post-intervention and are shown in Table 5. Within and between group changes in circulating cytokines due to the intervention were not statistically significant. However, the magnitude of the effect sizes indicated the difference of mean change in circulating cytokines between control and intervention group is not trivial. The absolute values of effect sizes for each

serum cytokine are small (0.2) to medium (0.5), meaning the two groups' mean changes differ by 0.2 to 0.5 standard deviations.

Serum Carotenoids

Serum lycopene, lutein, zeaxanthin, β -carotene, cryptoxanthin, xanthophylls and total carotenoids pre- and post-intervention for the experimental and control arm are shown in Table 4. There were no statistically significant differences between individual serum carotenoid concentrations and CV/GLV intake pre- and post-intervention. There were no significant differences of mean change in total serum carotenoid concentrations between control and experimental groups after intervention. The magnitude of most effect sizes, except β -carotene and xanthophylls, is small-to-large (0.2 to 0.8), suggesting non-trivial difference of mean change in lycopene, lutein, zeaxanthin, cryptoxanthin, and total carotenoids between groups.

DNA Methylation

There was little difference between pre- and post-treatment DNA methylation patterns within or between experimental and control groups. When all participants were combined, two sub-groups of either high lycopene or low IFN- γ (HL-LI) or low lycopene and high IFN- γ (LL-HI) following treatment were noted. Of those two groups, n=23 differentially methylated CpGs were associated with genes involved in metabolism, genetic stability, and regulation. Instead of changing with dietary intervention, hierarchical clustering revealed that DNA methylation is highly stable within participants.

Discussion

This pilot RCT is the first to assess the feasibility of an intervention to increase CV and GLV specifically (rather than overall fruit and vegetable intake) in post-treatment HNC survivors, regardless of stage.

The primary objective of the study was to determine the feasibility of implementing a dietary intervention rather than confirm efficacy. This pilot study consisted of 24 HNC survivors, n=12 per study arm. 12 participants per study arm has been deemed a sufficient sample size calculation for a pilot RCT⁴⁸. While there was no anticipation of identifying significant differences in biomarkers of interest in this study, it is highly possible that a longer, larger trial could greatly influence biomarker changes based on this studies preliminary effect size findings. An observed small- to large-effect sizes in the directions hypothesized for carotenoids and cytokines was noted, which will allow for the estimation of sample size for a larger RCT. There was not a sufficient volume of serum to perform lipid adjustment on serum carotenoids. However, lipid adjustment has previously been found to have little-to-no influence on the correlation between fruit and vegetable consumption and carotenoid concentrations⁴⁹.

To the best of the study team's knowledge, only two RCT's aimed at increasing fruit and vegetable intake in HNC survivors have previously been conducted^{14, 15}. While the other two studies enrolled early-stage survivors and focused on increasing overall fruit and vegetable intake with daily goals^{14, 15}, this study enrolled early and late-stage survivors and

focused on specific vegetables with weekly goals. Therefore, comparing the effectiveness of the dietary intervention on improvements in intake is difficult. However, all three RCTs were grounded in behavioral theories and led to a behavior change. This study provided more frequent telephone counseling sessions than the other two interventions. Late-stage survivors may likely suffer long-term symptoms impacting the ability and desire to eat, thus may benefit from more frequent contact and counseling techniques designed to help participants overcome symptoms while achieving food intake goals.

Although not statistically significant, study results suggest improvements of circulating cytokines and carotenoids due to the intervention. The magnitude of most effect sizes: all cytokines, small to medium (0.3 – 0.5) and carotenoids: lycopene, lutein, zeaxanthin, cryptoxanthin and total carotenoids, small-to-large (0.2 to 0.8), suggest a potential difference in mean change. Similarly, in a RCT of early-stage survivors (stage 1 and 2) of HNC by Cartmel et al., serum carotenoid biomarkers were greater in the experimental group as compared to the control group, although the difference in plasma carotenoids between the groups did not reach statistical significance¹⁴. Two reviews of RCTs support the role of the Mediterranean and DASH diets, which both emphasize consumption of green leafy vegetables⁵⁰, in lowering inflammatory cytokines, which are consistent with the results of this study^{51, 52}.

There were no observed differences in pre- and post-intervention DNA methylation patterns nor between experimental and control groups. Hierarchical clustering revealed DNA methylation was highly stable within participants, corroborating past findings that time-dependent intra-person variation in DNA methylation is less than inter-person variation⁴¹. Previous interventions have demonstrated variable impact on circulating DNA methylation^{53, 54}. Dietary interventions have generally focused on nutrients such as essential fatty acids^{55, 56}, folate^{57, 58} and B12⁵⁹. Changes in DNA methylation were observed in studies lasting from eight weeks⁵⁴ to two years⁵⁸. The short, 12-week, duration and small sample size of the current study are potential reasons a treatment effect was not noted. While the aforementioned dietary intervention studies used supplements^{55–59}, this study provided dietary counseling to promote a behavioral change in usual dietary intake. Thus, it is possible participants were not as adherent or did not receive high enough doses of nutrients in comparison to past interventions. In a cross-sectional study, folate, B12, vitamin A, and CV intake were associated with DNA methylation patterns in HNC patients¹⁶. However, in the present study, DNA methylation was measured in blood samples from HNC survivors, rather than tumor samples from HNC patients. Thus, further experimentation should aim to understand the implications of circulating DNA as a biomarker of current health and future outcomes.

Despite these insignificant results, vegetable intake was self-reported for both groups and based on only two measurements for the control group, baseline and 12-week follow-up. The experimental group reported the amount of vegetables consumed weekly, thus, the experimental group intake may not be accurately reflected from the 12-week follow-up alone (ex. ill, traveling, vacation) and could potentially influence the analysis. Therefore, there is a lack of evidence to conclude the experimental group consumed more total

vegetables as compared to the control group. However, the effect sizes suggest that a change in vegetable consumption did occur between groups.

There were several limitations of the study. Control group participants increased median intake of GLV by approximately two cups during the study, however this was not a statistically significant increase ($p=0.52$). A limitation of the study included the inability to blind study participants to the intervention. The control group was aware the intervention focused on increasing intakes of CV and GLV and thus may have altered behavior and created a Hawthorne effect⁶⁰. This “priming” may have been enough to lead to a behavior change, which is quite remarkable. Findings such as this have been documented in other behavioral intervention studies and are important considerations for developing future trials^{61, 62}. Perhaps the current study was too nutrition-focused and limited our abilities to observe differences between groups. However, this study lacked statistical power in dietary biomarker measures and future studies should enroll a larger sample size, at least 188 participants, to increase effect size. Furthermore, economic considerations were not taken into account during the recruitment process. Therefore, participants in the experimental group may have experienced cost-prohibiting challenges in purchasing CV and GLV. However, given the rural, southeastern nature of the study, many participants reported having home gardens, furthering intervention adherence.

This intervention had several strengths including the successful retention and adherence rates. The enrollment rate of 25% is moderate for a dietary trial among cancer survivors. The intervention was delivered primarily via telephone counseling, likely enhancing participation due to the convenient, home-based approach, and improves the translational potential of the intervention to multiple sites. The next step of research is to identify intervention and research design factors perceived to increase the potential for the intervention to be implemented on a more widespread or “scaled up” basis^{63, 64}. For instance, distance was the top reason for non-study participation. Participants were asked to report to the southeastern, National Cancer Institute-designated Comprehensive Cancer Center to complete the baseline and follow-up phlebotomy, counseling, and survey questionnaires. Strategies to overcome distance as a barrier should be employed in future, larger trials. Some possibilities include: providing a traveling phlebotomist to provide home-based blood sample collection, higher incentives, travel reimbursement, or administering the study over multiple sites. A monetary incentive of \$25 for the two study visits was provided; however, the stipend may have been too low given distance between the patient’s home and cancer center often being substantial due to the rural community environment surrounding the study site.

Conclusion

In conclusion, there is a growing interest in improving cancer-related morbidity, mortality, and QOL through dietary modification. This feasibility trial is a first step in providing evidence needed to justify the incorporation of dietary protocols into HNC survivorship care plans. The methodology used in this study can be modified to strengthen the next trial phase. For instance, attention control groups should focus on a health intervention that is not directly nutrition-related. This trial provided valuable information on feasibility outcomes including accrual, retention, adherence, safety, and secondary outcomes including

inflammatory markers and carotenoids. A larger RCT is warranted to assess the efficacy of this dietary intervention on disease outcomes.

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Research Snapshot:**Research Question:**

Is implementing a 12-week randomized clinical trial (RCT) to increase cruciferous vegetables (CV) and green leafy vegetables (GLV) feasible in post-treatment head and neck cancer (HNC) survivors?

Key Findings:

A RCT dietary intervention of 24 post-treatment HNC survivors focused on the experimental group (n=12) consuming 2.5 cups/week CV and 3.5 cups/week GLV was a feasible method of testing the effects of increasing vegetable consumption in this survivor population.

Figure 1:

Flow Diagram for head and neck cancer (HNC) survivors in a cruciferous and green leafy vegetable 12-week randomized clinical trial at a southeastern, Comprehensive Cancer Center between January 2015 – September 2016

online only: Multivariable HRs and 95% CIs for mortality according to quartile of intake of food group servings¹⁹ in patients enrolled in the University of Michigan Head and Neck Specialized Program of Research Excellence (HN-SPORE) from January 2003 to December 2008 (n=542)^a

Table 1 –

Mortality				
Vegetable Type	Q1	Q2	Q3	Q4
Green leafy vegetables	1.0	1.28 (0.89, 1.84)	0.82 (0.57, 1.19)	0.68 (0.46, 1.01)
Cruciferous vegetables	1.0	0.60 (0.42, 0.87)	0.89 (0.60, 1.31)	0.53 (0.36, 0.78)
				P _{trend}
				0.02 *
				0.01 *

^a Adjusted for age, sex, tumor site, cancer stage, treatment, ACE-27 comorbidities, smoking, BMI, and total energy intake

* Indicates significance at p<0.05

Table 2:

Clinical, sociodemographic and behavioral characteristics of head and neck cancer survivors in a cruciferous and green leafy vegetable 12-week randomized clinical trial at a southeastern, Comprehensive Cancer Center between January 2015 – September 2016

Characteristic	Experimental Group	Control Group	Total (%)	P Value
	n = 12 (%)	n = 12 (%)		
Age^a : Mean $\pm$ SD [range], years	59.1 $\pm$ 7.95 [45–76]	58.9 $\pm$ 8.73 [44–72]	59 $\pm$ 8.17 [44–76]	0.98
Sex^b :				
Male	10 (83.3)	9 (75)	19 (79.2)	0.62
Female	2 (16.7)	3 (25)	5 (20.8)	
Time since treatment completion^b :				
< 3 years	6 (50)	6 (50)	12 (50)	1.00
3 years	6 (50)	6 (50)	12 (50)	
Race^{bc} :				
Black/ African-American	2 (16.7)	1 (8.3)	3 (12.5)	0.51
White	10 (83.3)	10 (83.3)	20 (83.3)	
Native Hawaiian or Pacific Islander	0 (0)	1 (8.3)	1 (4.2)	
Education^b :				
High School	4 (33.3)	5 (41.7)	9 (37.5)	0.67
Some college or more	8 (66.7)	7 (58.3)	15 (62.5)	
Yearly household income^b : (dollars/year)				
<\$20,000	2 (16.7)	2 (18.2) ^d	4 (17.4)	0.35
\$20,000 - \$34,999	2 (16.7)	2 (18.2) ^d	4 (17.4)	
\$35,000 - \$49,999	3 (25.0)	0 (0.0) ^d	3 (13.0)	
\$50,000	5 (41.6)	7 (62.6) ^d	12 (52.2)	
Tumor Site^b :				
Hypopharynx	0 (0)	2 (16.7)	2 (8.3)	0.39
Larynx	4 (33.4)	4 (33.4)	8 (33.4)	
Oral Cavity	3 (25.0)	1 (8.3)	4 (16.7)	
Oropharynx	4 (33.4)	5 (41.7)	9 (37.5)	
Unknown Primary	1 (8.3)	0 (0)	1 (4.2)	
Cancer Stage^b :				
Stage 1	3 (25.0)	1 (8.3)	4 (16.6)	0.59
Stage 2	2 (16.6)	1 (8.3)	3 (12.5)	
Stage 3	3 (25.0)	4 (33.3)	7 (29.2)	

Characteristic	Experimental Group	Control Group	Total (%)	P Value
	n = 12 (%)	n = 12 (%)		
Stage 4	4 (33.3)	6 (50.0)	10 (41.7)	
Treatment^b:				
No Radiation	3 (12.5)	3 (12.5)	6 (25.0)	1.00
Radiation	9 (37.5)	9 (37.5)	18 (75.0)	
Smoking Status^b:				
Current	1 (8.3)	1 (8.3)	2 (8.3)	0.88
Former	8 (66.7)	9 (75)	17 (70.1)	
Never	3 (25)	2 (16.7)	5 (20.8)	
Alcohol Consumption^b:				
Current	6 (50)	7 (58.4)	13 (54.2)	0.91
Former	5 (41.7)	4 (33.3)	9 (37.5)	
Never	1 (8.3)	1 (8.3)	2 (8.3)	
Baseline BMI^a: Mean $\pm$ SD [range], kg/m ²	28.2 $\pm$ 5.17 [21.7–40.6]	27.9 $\pm$ 7.28 [18.5–40]	28.1 $\pm$ 6.05 [18.5–40.6]	0.92
Follow-up BMI^a: Mean $\pm$ SD [range], kg/m ²	28.0 $\pm$ 5.39 [22.4–41]	28.0 $\pm$ 6.76 [19.2–40]	28.0 $\pm$ 5.87 [19.2–41]	0.99

^a Student's t-test

^b Chi-square or Fisher's exact test was used as appropriate

^c No participants were Hispanic

^d n=11 responses

Table 3.

Study adherence of head and neck cancer survivors in a cruciferous and green leafy vegetable 12-week randomized clinical trial at a southeastern, Comprehensive Cancer Center between January 2015 – September 2016

	N (%)	N (%)	N (%)
	Total (N=23)	Control (N=12)	Experimental (N=11)
<i>Adherence to weekly telephone sessions^a</i>			
Mean adherence	90.4	95	85.8
<i>Mean experimental group adherence to weekly vegetable goals^a</i>			
Green Leafy Vegetables	-	-	74.4
Cruciferous Vegetables	-	-	67.2
Overall	-	-	70.8
<i>Median experimental vs control group vegetable intake baseline vs week 2–12 (cups/week)^b</i>			
	Baseline	Follow-up	Baseline
	Follow-up		
Green Leafy Vegetables	-	0	2
Cruciferous Vegetables	-	0	0
			3
<i>Mean experimental group vegetable intake baseline vs week 2–12 (cups/week)^b</i>			
Green Leafy Vegetables	-	-	0
Cruciferous Vegetables	-	-	0
			5.5
			3.5

^a Adherence was measured as completion of the weekly telephone sessions (for both groups) and self-reported weekly CV and GLV intake for the experimental group, irrespective of goal intake

^b Adherence to weekly goals assessed using data collected from weekly recalls and 24-hour diet recalls

Table 4:

Pre- vs. post-intervention vegetable intake between and within groups in head and neck cancer survivors at a southeastern, Comprehensive Cancer Center between January 2015 – September 2016

	Control Group				Experimental Group						
	Baseline	Follow-up			Baseline	Follow-up ^a					
Vegetables cups/week measured by 24-hour diet recalls and weekly recalls ^b	Mean (SD)	Mean (SD)	MPD ^c	Within-group p-value	Mean (SD)	Mean (SD)	MPD ^c	Within-group p-value	Between-group Difference of MPD	Between-group p-value	Cohen's d (effect size)
Cruciferous ^d	0.02 (0.05)	1.33 (2.39)	1.30	0.38 ^e	1.32 (1.94)	3.19 (2.24)	1.87	0.04 ^f	0.57	0.5	0.22
Green Leafy ^g	1.11 (1.76)	1.68 (1.65)	0.43	0.52 ^f	2.05 (2.61)	2.58 (2.08)	0.53	0.48 ^f	0.10	0.92	0.04
Total ^g	1.13 (1.79)	3.02 (2.64)	1.73	0.12 ^f	3.37 (4.01)	5.77 (4.02)	2.40	0.12 ^f	0.67	0.70	0.16

^aOne participant in experimental group missing follow-up

^bonly experimental group completed weekly records

^cdenotes 'mean paired difference'

^dWilcoxon rank-sum test performed

^eSign test

^fPaired t-test performed

^gTwo-sample t-test performed

Table 5:

Pre- vs. post-intervention serum cytokine and carotenoid biomarkers in head and neck cancer survivors at a southeastern, Comprehensive

	Control Group			Experimental Group				
	Baseline	Post-intervention		Baseline	Post-intervention			
	Mean (SD)	Mean (SD)	MPD ^a	Mean (SD)	Mean (SD)	MPD ^a		
Cytokine µg/mL								
C-reactive protein (CRP) ^c	3.76 (2.03)	3.58 (2.08)	-0.18	4.83 (6.24)	3.78 (3.93)	-1.37	0.88	-0.31
Interferon-γ (IFN-γ) ^c	9.20(5.41)	8.85 (4.93)	-0.35	8.24 (5.69)	10.48 (9.70)	2.08	0.83	0.38
Interleukin-1(3 (IL-1β) ^c	0.04 (0.02)	0.06 (0.04)	0.02	0.03 (0.02)	0.03 (0.02)	0.00	0.78	-0.46
Interleukin-6 (IL-6) ^c	1.05 (0.61)	1.26 (1.14)	0.21	0.79 (0.51)	0.76 (0.58)	-0.04	0.49	-0.40
Tumor necrosis factor-α (TNF-α) ^c	2.50 (0.60)	2.62 (0.56)	0.13	2.73 (1.08)	2.68 (1.46)	-0.08	0.20	-0.32
Carotenoids µg/mL								
Lycopene ^e	0.46 (0.23)	0.42 (0.21)	-0.03	0.42 (0.20)	0.50 (0.25)	0.08	0.08	0.77
Lutein ^e	0.14 (0.11)	0.14 (0.08)	0.001	0.09 (0.04)	0.11 (0.05)	0.01	0.53	0.26
Zeaxanthin ^e	0.03 (0.01)	0.02 (0.01)	-0.003	0.02 (0.01)	0.02 (0.01)	0.00	0.30	0.51
p-carotene ^e	0.20 (0.20)	0.17 (0.16)	-0.02	0.31 (0.40)	0.31 (0.42)	-0.01	0.34	0.13
Cryptoxanthin ^e	0.05 (0.04)	0.06 (0.07)	0.002	0.05 (0.05)	0.05 (0.04)	-0.006	0.65	-0.20
Xanthophylls ^{e,f}	0.22 (0.14)	0.22 (0.15)	0.00	0.16(0.07)	0.17 (0.08)	0.01	0.52	0.13
Total Carotenoids ^{e,f}	0.87 (0.39)	0.81 (0.41)	-0.06	0.89 (0.59)	0.98 (0.59)	0.08	0.13	0.66

^aDenotes 'mean paired difference'^bp-value is calculated from Wilcoxon Rank-Sum Exact test since distributions are not normal^cTo convert µg/mL CRP to nmols/L, multiply µg/mL by 43.4308. To convert µg/mL IFN-γ to nmols/L, multiply µg/mL by 51.6849. To convert µg/mL IL-1β to nmols/L, multiply µg/mL by 32.5250. To convert µg/mL IL-6 to nmols/L, multiply µg/mL by 42.1639. To convert TNF-α, µg/mL to nmols/L, multiply µg/mL by 38.9960.^dTwo-sample t-test for lycopene, lutein, zeaxanthin and total carotenoids; Wilcoxon rank-sum test for β-carotene, cryptoxanthin and xanthophylls

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To convert $\mu\text{g/mL}$ Lycopene to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.8626. To convert $\mu\text{g/mL}$ Lutein to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.7579. To convert $\mu\text{g/mL}$ Zeaxanthin to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.7577. To convert $\mu\text{g/mL}$ β -carotene to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.8625. To convert $\mu\text{g/mL}$ Cryptoxanthin to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.8086. To convert $\mu\text{g/mL}$ Xanthophylls to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.7741. To convert $\mu\text{g/mL}$ Total Carotenoids to $\mu\text{mols/L}$, multiply $\mu\text{g/mL}$ by 1.8625.

_f Xanthophylls was determined as the sum of Lutein, Zeaxanthin, and Cryptoxanthin. Total carotenoids was measured and represents the sum of carotenes and lycopene.