Minimal Impact of Nutrition Education and Fruit and Vegetable Consumption on Biomarkers of Inflammation and Oxidative Stress

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Authors’ contributions

This work was carried out in collaboration between all authors. Author MGW designed the study, wrote the protocol, conducted the research, performed the statistical analysis, wrote the first draft of the manuscript, and had primary responsibility for the final content. Author YR supervised the research and had primary responsibility for the final content. Author KHH conducted the research. Author EHBS provided assistance with statistical analysis and reviewed/edit ed the manuscript. Author DT reviewed/edited the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: To determine the effectiveness of a community-based fruit and vegetable education program and provision of fruits and vegetables on consumption of fruits, vegetables, antioxidants, and changes in biomarkers of inflammation and oxidative stress among overweight and obese adults.

Study Design: Randomized controlled design.

Place and Duration of Study: North Dakota State University, Fargo, North Dakota; 14 weeks.

Methodology: Forty-seven adults (31 women; 45.9 ± 11.8 y; body mass index 32.7 kg/m²) were randomly assigned to one of three intervention groups. The control group received no intervention, the education group attended weekly nutrition education lessons, the fruit and vegetable group attended weekly nutrition education lessons and received one serving of fruits and two servings of vegetables per day for 10 weeks. Fasting blood was drawn and consumption of fruits, vegetables,
and antioxidants was assessed using three-day food records.

**Results:** Increased consumption of fruit from pre-to post-test was indicated among fruit and vegetable group participants, $P = .01$, and among education group participants, although this difference was not significant, $P = .11$. In contrast, a significant decrease in fruit servings consumed from pre-to post-test for control group participants was observed, $P = .02$. Vegetable consumption was the same for control group participants, decreased by 0.3 serving among education group participants, and increased by 0.4 serving among fruit and vegetable group participants. No significant differences in plasma TNF-α, TBARS, or CRP concentrations from pre-to post-test were indicated among the three groups, although the largest decrease was observed among fruit and vegetable group participants, $P = .07$.

**Conclusion:** Changes in fruit and vegetable consumption among participants were minimally associated with improvements in inflammation and oxidative stress biomarkers. Adequate and varied fruit and vegetable consumption is recommended to aid in the prevention and regulation of inflammation and oxidative stress.

**Keywords:** Antioxidant; overweight; obesity; tumor necrosis factor-alpha; C-reactive protein.

**ABBREVIATIONS**

BMI (body mass index); CON (control group); CRP (C-reactive protein); EDUC (education group); FV (fruit and vegetable group); MDA (malondialdehyde); TBARS (thiobarbituric acid reactive substances); TNF-α (tumor necrosis factor-alpha).

**1. INTRODUCTION**

In the U.S., more than 60% of adults are overweight or obese [1]. The rising rates of overweight and obesity are particularly concerning because of the numerous health conditions associated with excess weight, including cardiovascular disease, type 2 diabetes mellitus, and certain cancers [2,3]. An underlying mechanism believed to contribute to these detrimental health outcomes is the inflammatory process [4]. Excess adipose tissue contributes to disruption of metabolic homeostasis [5] and leads to an accumulation of free radicals [6]. The presence of free radicals perpetuates the secretion of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), which induce the release of C-reactive protein (CRP) from the liver [7-9]. The increased concentration of inflammatory cytokines and CRP result in a state of chronic, obesity-induced, low-grade inflammation, which is associated with increased disease risk [10]. Fortunately, the body is able to prevent accumulation of free radicals via antioxidants, which donate electrons in order to stabilize free radicals and prevent oxidative damage [11].

While some antioxidants are present in the body, others such as vitamins and minerals must be consumed through the diet [12,13]. Plant foods, including fruits and vegetables, have a substantially higher antioxidant content compared to meat, fish, and other animal-based foods [14]. Because of their excellent nutrient profiles, consumption of fruits and vegetables is widely encouraged [15-17]. However, according to the CDC [18], less than 15% of U.S. adults consume the recommended five or more daily servings of fruits and vegetables, with rates decreasing among some adult cohorts. Specifically, among overweight and obese adults, fruit and vegetable consumption was observed to significantly decrease from 22.6% to 21.5% over an eight-year period [19].

Previous studies have assessed the influence of educational interventions on fruit and vegetable consumption [20-22], antioxidant consumption [23,24], role of antioxidants in oxidative stress [14,25-27], and the correlation between fruit and vegetable consumption and inflammation in healthy individuals [16,17,28] and found favorable results. However, research examining the effects of both nutrition education and provision of fruits and vegetables on biomarkers of inflammation and oxidative stress is lacking. Therefore, this study was designed to determine the effectiveness of a community-based fruit and vegetable education program and provision of fruits and vegetables on consumption of fruits, vegetables, and antioxidants, and changes in biomarkers of inflammation and oxidative stress among overweight and obese adults. Goals were
to increase participants’ consumption of fruits and vegetables and evaluate subsequent changes in antioxidant consumption and inflammatory and oxidative stress biomarkers.

2. MATERIALS AND METHODS

2.1 Participants

A convenience sample of adults with a body mass index (BMI) of 25 kg/m² or greater was recruited via flyers and email notices from two neighboring communities with a combined population of approximately 135,000 residents. Exclusion criteria included being under 18 years of age, a current smoker, having a BMI lower than 25 kg/m², being pregnant or lactating, or having a history of bariatric surgery. Study protocol was approved by the North Dakota State University Institutional Review Board prior to implementation and all participants provided written informed consent.

2.2 Procedures

The study consisted of three phases over a 14-week period: pre-test (2 weeks), intervention (10 weeks), and post-test (2 weeks). Of the 67 participants originally enrolled in the study, 46 completed both pre- and post-test food records. Of these, 8 participants were in the control (CON) group, 15 were in the education (EDUC) group, and 23 were in the fruit and vegetable (FV) group. At pre-test, self-reported demographic information was collected. Participants were also asked to report medical history and use of prescription and over the counter medications, as inflammatory conditions or use of anti-inflammatory medications needed to be accounted for prior to data analysis. Information about habitual fruit and vegetable intake was also assessed at both pre- and post-test, using modified versions of two semi-quantitative food frequency questionnaires developed by nutrition experts at The University of Georgia [29].

Food and nutrient intake at pre- and post-test were assessed using a three-day food record. Validity of food records in assessment of energy and nutrient intake has been demonstrated in previous studies of adults [30-32]. Three-day food records require less participant burden, result in improved compliance, and provide accuracy comparable to seven-day food records [33]. Participants were instructed to record everything they ate or drank, as it was consumed, over the course of two weekdays and one weekend day. Data collected from the food records were entered by a registered dietitian and analyzed using The Food Processor® (ESHA Research, Salem, OR). To ensure validity, all data were entered by the same individual. Participant’s age, gender, height, weight, and physical activity level were also entered into the program to allow for comparison with the U.S. Dietary Guidelines [34] for servings from each food group.

During pre- and post-test, anthropometric data were collected, including height, weight, waist circumference, and BMI. In addition, a total of 30 mL of venous whole blood sample was drawn after a 12-hour overnight fast. Blood samples at both pre- and post-test were collected from a total of 40 participants (6 in the CON group, 12 in the EDUC group, and 22 in the FV group). All blood draws were scheduled and performed by a phlebotomist at a local hospital. Within 30 minutes of blood being drawn, EDTA-plasma samples were separated from whole blood by laboratory staff at the hospital and frozen immediately at -80°C. Samples were transferred from the local hospital to the university in ice and stored in a freezer at -80°C.

Following pre-test, participants were randomly assigned to one of three intervention groups: CON, EDUC, or FV. Participants assigned to the CON group received no intervention but were given the option of receiving the fruit and vegetable information provided to other participants after the intervention was over. Participants in the EDUC group received weekly information about the antioxidant content of fruits and vegetables, the role of antioxidants in the inflammatory process, and the current recommendations for fruit and vegetable consumption. Participants assigned to the FV group received the same information as participants in the EDUC group. In addition, they received recommendations for incorporating fruits and vegetables into their current meal plan and samples of fruits and vegetables. The samples included fresh, frozen, or canned fruits and vegetables and were provided to participants at the weekly nutrition education sessions. The quantity provided to each FV group participant was the equivalent of one serving of fruit and two servings of vegetable per day for a week because on average, Americans fail to meet the recommended daily fruit and vegetable servings by these amounts [35]. Servings provided were
consistent with the serving sizes listed in the 2010 U.S. Dietary Guidelines for Americans [34]. Participants in the FV group were encouraged to consume these fruits and vegetables as a supplement to their usual fruit and vegetable intake.

Nutrition lessons, approximately 30 minutes in duration, were provided weekly by licensed, registered dietitians at a local university. Curriculum materials, developed by nutrition experts at The University of Georgia [29,36], were used for the nutrition lessons. This curriculum was chosen because it was consistent with the purpose of the study, had been used for previous research [29,37,38], and was readily available to the public via the Internet [36]. Curriculum materials included scripts for each lesson, handouts describing how to purchase and store fruits and vegetables, lists of the nutrient content of various fruits and vegetables, and suggestions for incorporating fruits and vegetables into meals and snacks.

After conclusion of the 10-week intervention, post-test was initiated in order to evaluate changes in consumption of fruits, vegetables, and antioxidants as well as changes in biomarkers of inflammation and oxidative stress. Intake of fruits, vegetables, and antioxidants was again assessed using three-day food records.

2.3 Biochemical Analysis

Once all samples were collected, samples were thawed to room temperature and plasma CRP and TNF-α concentrations were measured according to manufacturer instructions using ELISA kits (CRP ELISA kit, Cayman Chemical Company, Ann Arbor, MI; TNF-α UltraSensitive ELISA Kit, Invitrogen Corporation, Camarillo, CA). Oxidative stress was measured using a TBARS kit (Cayman Chemical Company, Ann Arbor, MI). Microtiter plates were read by a microtiter plate reader (ELx 808 IU, Bio-Tek Instruments, Inc., Winooski, VT) at 550 nm. All standards and samples were run in duplicate for the CRP and TNF-α assays and in triplicate for the TBARS assay.

Results of plasma CRP concentrations were expressed as mg CRP/L and results of TNF-α were expressed as pg TNF/mL. Results of the oxidative stress analysis reflected the concentration of malondialdehyde (MDA), which is a byproduct of lipid peroxidation. Results were expressed as MDA equivalents (nmol MDA/mL plasma), with higher values indicating a higher degree of oxidative stress.

2.4 Statistical Analysis

All statistical analyses were performed using PASW version 18.0 (SPSS Inc., Chicago, IL). Chi square analyses were used to assess differences among intervention groups in regard to demographics. Descriptive statistics were generated for all participants completing both pre- and post-test. Paired sample t-tests were used to evaluate mean differences in weight, BMI, CRP, TNF-α, and TBARS concentrations from pre- to post-test for all participants and within the three groups. Pearson correlation analysis was used to evaluate relationships between changes in fruit and vegetable consumption and changes in biomarkers of inflammation and oxidative stress from pre- to post-test. Finally, one-way ANOVA was used to assess if nutrition education about antioxidant properties and the antioxidant content of fruits and vegetables, with and without provision of fruit and vegetable samples, had a differential effect on CRP, TNF-α, and TBARS concentrations from pre- to post-test compared to no intervention (CON group).

3. RESULTS

Participants were predominantly female (66.0%), white (91.5%), married or partnered (78.7%), and reported an annual household income in the range of $50,000-$99,999 (44.7%). Chi square tests for independence did not indicate differences in proportions in regard to gender, ethnicity (white, non-white), relationship status (married, other), or reported annual household income ($≤39,999, ≥$40,000) between intervention groups. The mean BMI among all participants at pre-test was 32.7 kg/m². Results of paired sample t-tests indicated no significant differences in weight and BMI from pre- to post-test (data not shown).

Changes in use of anti-inflammatory medications from pre- to post-test were accounted for and plasma concentrations beyond two standard deviations above or below the mean were eliminated prior to data analysis. One-way ANOVA indicated no significant differences in antioxidant consumption or CRP, TNF-α, or TBARS concentrations between intervention groups at pre-test.
Results of paired sample t-tests failed to indicate significant mean differences in inflammation and oxidative stress biomarkers from pre- to post-test (Table 1). Among all participants, mean TNF-α concentration decreased to an undetectable concentration at post-test. No significant differences in plasma TBARS concentrations from pre- to post-test were observed among CON, EDUC, or FV group participants. While differences in CRP concentrations between pre- to post-test were not significantly different for participants in any of the intervention groups, the largest decrease was observed among FV group participants, t(19) = -1.96, P = .07.

Results of paired sample t-tests indicated a significant increase in consumption of fruit from pre- to post-test among FV group participants, t(22) = 2.89, P = .01 (Table 2). An increase in fruit servings consumed at post-test compared to pre-test was also noted among EDUC group participants, but this increase did not represent a significant difference, t(14) = 1.72, P = .11. In contrast, results showed a significant mean decrease in the number of fruit servings consumed from pre- to post-test for CON group participants, t(7) = -2.89, P = .02. No significant differences in vegetable consumption existed from pre- to post-test for participants in any of the intervention groups. Vegetable consumption was the same for CON group participants, decreased by 0.3 serving among EDUC group participants, and increased by 0.4 serving among participants in the FV group.

Results of paired sample t-tests failed to indicate significant mean differences in antioxidant consumption from pre- to post-test among the total sample and the different intervention groups (Table 2). Despite absence of significant differences, a consistent increase in consumption of beta carotene from pre- to post-test was observed among all groups. In fact, increased consumption of beta carotene among FV group participants trended towards significance, t(22) = 2.04, P = .05. Changes in consumption of other antioxidants among the different intervention groups were inconsistent. Increases in vitamin C consumption were found among participants in the CON and FV group but not in the EDUC group. Consumption of vitamin E and selenium increased among CON group participants but decreased among EDUC and FV group participants.

Results of Pearson correlation analyses indicated moderate positive correlations between fruit consumption and consumption of vegetables (r = 0.30, P = .03) and beta carotene (r = 0.37, P = .01) at post-test. A strong positive correlation was indicated between fruit and vitamin C consumption (r = 0.57, P<.01). In addition, a moderate negative correlation existed between fruit consumption and CRP concentration (r = -0.41, P = .01). Significant positive correlations also existed between vegetable consumption and beta carotene (r = 0.56, P<.01) and selenium (r = 0.30, P = .03) consumption at post-test. Positive correlations were also indicated between the various antioxidants (Table 3). However, no significant relationships were found between post-test consumption of fruits, vegetables, and antioxidants, and TBARS concentration. TNF-α was not included in the correlation analysis due to undetectable concentrations at post-test.

Table 1. Mean concentrations of inflammation and oxidative stress biomarkers at pre- and post-testa

| Variable          | Control (n = 6)    | Education (n = 12) | Fruit and vegetable (n = 20) |
|-------------------|--------------------|--------------------|-----------------------------|
|                   | Pre | Post | Pre | Post | Pre | Post |
| CRP mg/L          | 4.34 ± 1.16 | 4.31 ± 1.03 | 2.34 ± 0.64 | 2.11 ± 0.75 | 2.89 ± 0.84 | 1.52 ± 0.34 |
| TNF-α pg/mL       | 5.90 ± 1.13 | n.d. | 5.14 ± 0.91 | n.d. | 5.94 ± 0.66 | n.d. |
| TBARS nmol/mLb    | 13.25 ± 1.34 | 7.94 ± 2.06 | 16.54 ± 3.18 | 11.71 ± 2.58 | 13.63 ± 1.14 | 10.56 ± 1.41 |

| Data are means ± standard error. n.d. = not detected; CRP = C-reactive protein; TNF-α = tumor necrosis factor-alpha, TBARS = thiobarbituric acid reactive substances |
| Difference in total sample size due to two outliers being eliminated from fruit and vegetable group (N = 36). |
| nmol/mL of malondialdehyde equivalents |
Table 2. Mean daily servings of fruits, vegetables, and antioxidants consumed at pre-test and post-test

| Variable               | Control (n = 8) | Education (n = 15) | Fruit and vegetable (n = 23) |
|------------------------|----------------|-------------------|-----------------------------|
|                        | Pre | Post | Pre | Post | Pre | Post |
| Total fruits, svgs\(^a\) | 1.4 ± 0.3 | 0.8 ± 0.2 | 1.2 ± 0.3 | 1.5 ± 0.3 | 1.5 ± 0.2 | 2.2 ± 0.2 |
| Total vegetables, svgs | 1.4 ± 0.3 | 1.4 ± 0.2 | 1.8 ± 0.3 | 1.5 ± 0.2 | 1.6 ± 0.2 | 2.0 ± 0.2 |
| Beta carotene, mg\(^b\) | 1.3 ± 0.6 | 2.2 ± 1.0 | 1.7 ± 0.7 | 2.3 ± 0.8 | 2.1 ± 0.4 | 3.7 ± 0.7 |
| Vitamin C, mg          | 56.8 ± 11.7 | 80.8 ± 21.5 | 103.5 ± 24.1 | 90.7 ± 14.9 | 90.5 ± 13.9 | 115.0 ± 10.8 |
| Selenium, µg           | 4.9 ± 1.6 | 5.1 ± 2.1 | 5.7 ± 1.3 | 4.7 ± 1.4 | 4.2 ± 0.7 | 3.9 ± 0.6 |

\(^a\)Data are means ± standard error. Test statistics are t statistics. Different from pre, P<.05. svgs = servings; mg = milligrams; µg = micrograms.

\(^b\)Mean number of servings consumed derived from average daily consumption as recorded on three-day food records. Three-day food record data analyzed using The Food Processor\(^\text{®}\) (ESHA Research, Salem, OR).

\(^c\)Mean daily consumption of antioxidants was derived from average consumption as recorded on three-day food records. Three-day food record data were analyzed using The Food Processor\(^\text{®}\) (ESHA Research, Salem, OR).

Finally, results of one-way ANOVA to evaluate differences in biomarkers of inflammation and oxidative stress from pre- to post-test revealed no significant differences between the three intervention groups regarding changes in CRP or TBARS concentrations over time.

4. DISCUSSION

This study investigated the effects of a 10-week nutrition education program and provision of fruits and vegetables on fruit and vegetable consumption and subsequent changes in biomarkers of inflammation and oxidative stress among overweight and obese adults. Results indicated that at pre-test, participants were obese, with a mean BMI of 32.7 kg/m\(^2\) [39], and experiencing a moderate degree of inflammation and high degree of oxidative stress as evidenced by mean plasma concentrations (CRP = 2.91 mg/L; TNF-α = 5.70 pg/mL; TBARS = 14.50 nmol/mL) [40,41]. In addition, participants’ mean weekly consumption of fruits and vegetables was below the current recommendations to consume five or more daily servings [18]. Inadequate consumption of fruits and vegetables is particularly concerning because research has demonstrated that fruit and vegetable consumption is associated with improved antioxidant consumption [23]. Adequate consumption of antioxidants, which include beta carotene, vitamin C, vitamin E, and selenium, has been associated with a reduction in both inflammation and oxidative stress [11,26,27,42]. Participants who received nutrition education and fruit and vegetable samples reported increased consumption of fruits, but not vegetables. Alternatively, a decrease in fruit consumption from pre- to post-test was noted among CON group participants. Such a decrease in consumption was due to seasonal changes, transitioning from fall to winter, and absence of an intervention effect of education on benefits of fruit consumption, which was not provided to CON group participants. In addition, small increases in vegetable intake were only noted among participants in the FV group. These findings suggest that provision of fruits and vegetables was effective at increasing fruit and vegetable consumption. This is the first study to examine the effects of providing fruits and vegetables on consumption patterns of...
overweight and obese adults. However, this does support previous findings, which suggest availability and exposure to fruits and vegetables are possible determinants of fruit and vegetable consumption [43,44].

Both fruit and vegetable consumption at post-test were related to intake of beta carotene at post-test. In addition, post-test fruit consumption was related to vitamin C intake and post-test vegetable consumption was related to selenium intake. These findings are in agreement with existing literature that identifies fruits and vegetables as key sources of antioxidants, including beta carotene and vitamin C, in the diet [23]. While the positive relationships between fruit, vegetable, and antioxidant consumption are encouraging, participants’ overall mean consumption of fruits (1.7 servings) and vegetables (1.7 servings) following the intervention was below the recommended two or more daily servings of fruits and three or more daily servings of vegetables. Failure to consume an adequate quantity of fruits and vegetables likely hindered participants’ consumption of beneficial antioxidants.

Despite minimal changes in fruit, vegetable, and antioxidant consumption from pre- to post-test, a negative relationship between fruit consumption and CRP concentration at post-test was indicated, which is consistent with previous findings in healthy individuals [16,17,28]. Similar relationships with TBARS concentrations were not found following correlation analyses. Changes in CRP, TNF-α, and TBARS concentrations over time and among different intervention groups were noted, despite absence of significant weight changes. TNF-α concentrations in all groups were within the normal high sensitivity reference range (1.2-15.3 pg/mL) [45] at pre-test, and were undetectable at post-test, meaning all concentrations were below the minimum detectable concentration (0.009 pg/mL) of the ELISA kit used. Such decreases in TNF-α concentration may be attributed to increases in fruit, vegetable, or antioxidant consumption from pre- to post-test among participants, although no one variable was consistently higher at post-test among all three groups. Decreases in CRP concentrations were greatest among participants in the FV group, which suggests a possible benefit of provision of fruits and vegetables on decreased inflammation. Previous research has suggested associations between dietary fiber [16], omega-3 fatty acids [26], and reductions in markers of inflammation.

While intake of these dietary components was not a focus of the present study, the potential of these factors to impact markers of inflammation did exist.

Similar outcomes were observed in regard to oxidative stress. Trends toward a significant decrease in TBARS concentration were noted among FV and CON group participants more so than EDUC group participants. While a significant relationship was not observed, increased consumption of vitamin C among these two groups compared to decreased concentration in the EDUC group serves as a possible explanation for these results. As an antioxidant, vitamin C is especially effective at protecting against lipid peroxidation [11,46] and thus, helps to explain why decreased TBARS concentrations were observed among the groups with higher vitamin C consumption.

While changes in CRP, TNF-α, and TBARS concentrations were observed from pre- to post-test, these changes were not significantly different among participants who received the intervention compared to those who did not. These findings are contradictory to literature indicating associations between increased fruit and vegetable consumption and improvements in biomarkers of inflammation and oxidative stress [16,17,28]. A possible reason for lack of differences between participants in the EDUC and FV groups and those in the CON group may be due to the unequal sizes of the intervention groups and subsequent lack of statistical power.

The strength of this study was the randomized intervention design conducted in a sample of overweight and obese adults. A limitation was the unequal sizes of the intervention groups. More participants were purposely included in the EDUC and FV groups compared to CON group in anticipation of attrition, but the final number of participants in the CON group fell short of the required sample size and may have prevented detection of significant differences. In regard to biochemical analysis of plasma samples, TNF-α concentrations at post-test were below the specificity of the ELISA kit used and therefore, undetectable. This prevented evaluation of changes from pre- to post-test and among the different intervention groups. A possible solution would be to measure TNF-α expression by immunoblotting techniques or gene expression instead of ELISA. In addition, a longer intervention may have allowed for detection of differences.
Results of the present study highlight a need for additional research, including long-term studies, evaluating the effects of nutrition education interventions with provision of fruits and vegetables on changes in consumption of fruits, vegetables, and antioxidants, and biomarkers of inflammation and oxidative stress in overweight and obese adults. Previous research suggests that improvements in fruit and vegetable consumption could potentially contribute to improved antioxidant intake [23,24], reduced inflammation and oxidative stress [16,17,25,26], and ultimately decreased chronic disease risk. Therefore, additional research that controls for confounding variables and includes long-term follow-up is warranted.

5. CONCLUSION

This intervention to provide nutrition education and fruit and vegetable samples to overweight and obese adults at risk for chronic disease resulted in increased fruit consumption, but inconsistent changes in vegetable and antioxidant consumption. In addition, minimal improvements in biomarkers of inflammation and oxidative stress were noted. Other factors such as changes in anthropometrics, physical activity, and macronutrient intake may have impacted these measures [47-49]. Despite the present findings, evidence suggests that a balanced diet, including antioxidant-rich fruits and vegetables, is beneficial in helping individuals maintain adequate antioxidant concentrations in order to aid in the prevention and regulation of inflammation and oxidative stress [23,50]. Therefore, adequate and varied consumption of fruits and vegetables is recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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