Low-Fat, High Fruit and Vegetable Diets and Weight Loss Do Not Affect Biomarkers of Cellular Proliferation in Barrett Esophagus

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Materials and Methods

Participants. We recruited participants from May 1996 through July 1999. Most (82%) were recruited from

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Abstract

Risk factors for esophageal adenocarcinoma include obesity, high fat intake, and low consumption of fruits and vegetables. This trial tested whether an intervention to reduce these risk factors in patients with Barrett esophagus, a preneoplastic condition for esophageal adenocarcinoma, could reduce biomarkers of cellular proliferation and, by inference, the risk of neoplastic progression. Eighty-seven men and women with Barrett esophagus were randomized to an intensive dietary intervention or control group. At baseline, 18 and 36 months after intervention, biopsies were obtained at 2-cm intervals throughout the length of the Barrett segment. Ki67/DNA content flow cytometry was used to assess (a) % Ki67-positive proliferating diploid G1 cells, (b) % total Ki67-positive proliferating cells, (c) presence of aneuploidy, and (d) presence of >6% of cells in the 4N (G2/tetraploid) fraction of the cell cycle. We also assessed re-epithelialization and length of the Barrett segment, reflux symptoms, and medication use. The intervention effects for energy, fat, fruits and vegetables, and weight were, respectively, −314 kcal, −12.2% energy, 1.8 servings/d, and −4.0 kg at 18 months (all P < 0.005) and were smaller but remained significant at 36 months. There were no significant effects of the intervention on any biomarker of cellular proliferation. The intervention effects ± SE for mean %G1 Ki67+ cells were 0.98 ± 1.58 at 18 months and 1.79 ± 1.31 at 36 months; the relative risks (95% confidence interval) for developing >6% of cells in 4N were 0.5 (0.1-2.6) at 18 months and 0.75 (0.2-3.1) at 36 months. A single control participant developed aneuploidy. There were no significant effects on re-epithelialization, segment length, or reflux medication use. We conclude that substantial dietary change has no short-term effects on biomarkers of cellular proliferation in Barrett esophagus or on clinical observations of the Barrett segment. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2377–83)

Introduction

Barrett esophagus is a condition arising from chronic gastroesophageal reflux disease in which the normal stratified squamous epithelium of the distal esophagus is replaced by intestinal metaplasia (1). The true prevalence of Barrett esophagus is not known, but there may be as many as two million cases in the United States (2). Barrett esophagus is a premalignant condition, and among persons with Barrett esophagus, ~0.5% to 1% per year develop esophageal adenocarcinoma (3, 4). Since 1974, the incidence of esophageal adenocarcinoma in the United States has increased >400% in White males and 300% in White females (5-7), and it is now among the 15 most common cancers. Although studies are not entirely consistent, there is strong evidence that risk factors for esophageal reflux disease and esophageal adenocarcinoma are similar and include smoking, obesity, and high fat intake (8-12). Aspirin and other nonsteroidal anti-inflammatory drugs are associated with reduced risk of adenocarcinoma (13-15). Clinical management of persons with Barrett esophagus is based on pharmacologic treatment of gastroesophageal reflux and periodic endoscopic surveillance (1). As part of this surveillance, we have used DNA content flow cytometry to identify characteristics of Barrett epithelium that are associated with progression to cancer. Two of the strongest biomarkers predicting progression are the presence of aneuploid cell populations and the presence of >6% of cells in the 4N (G2/tetraploid) fraction of the cell cycle, which are associated with relative risks for cancer of 5.0 and 7.5, respectively (16). Among persons with Barrett esophagus, obesity (in particular high waist/hip ratio) is associated with increased risk of aneuploidy and elevated 4N fractions in Barrett epithelium (14, 17). Given the similarity between population studies finding associations of obesity and dietary risk factors with esophageal adenocarcinoma and the association of high waist/hip ratio with development of biomarker abnormalities in Barrett patients, we hypothesized that a dietary intervention designed to lower fat, increase fruit and vegetable consumption, decrease weight, and avoid foods triggering gastroesophageal reflux could be a useful adjunct treatment for persons with Barrett esophagus. We report here the results of a randomized clinical trial to evaluate whether a diet low in fat, high in fruits and vegetables and promoting weight loss affects flow cytometric biomarkers of cellular proliferation in men and women with Barrett esophagus. We also examined whether the dietary intervention reduces reflux symptoms as well as the use of medications to manage these symptoms.

Materials and Methods

Participants. We recruited participants from May 1996 through July 1999. Most (82%) were recruited from...
community-based medical practices, and the remainder were participants in our ongoing Seattle Barrett Esophagus Study for the early detection of adenocarcinoma in Barrett Esophagus (18). Participants in the Seattle Barrett Esophagus Study receive periodic endoscopic evaluations that include both histologic evaluation of esophageal tissue and DNA content flow cytometry of esophageal biopsies (18). Patients with histologically confirmed intestinal metaplasia in the esophagus were eligible for this study if they were ages 40 to 79 years, had a minimum of 3-cm Barrett segment length, and had had an endoscopy within 4 months of randomization. Persons with a history of cancer or cardiovascular disease, a concurrent disease that would preclude dietary intervention such as diabetes, or for whom weight loss was not appropriate (body mass index < 26 kg/m² and waist/hip ratio < 0.95) were excluded. Furthermore, patients with evidence of advanced neoplastic progression in Barrett epithelium, defined histologically as high-grade dysplasia or by DNA content flow cytometry as presence of aneuploid cell populations or 4N fraction of ≥6%, were excluded because we believed that their disease was not likely to be influenced by dietary factors. Consent to conduct this research was obtained from the Human Subjects Review Committee at the University of Washington and the Fred Hutchinson Cancer Research Center.

**Recruitment, Randomization, and Intervention.** Participants were initially contacted by phone and screened for eligibility and interest. Eligible participants completed a 2-week run-in period, consisting of maintaining a 4-day diet record and 7-day symptom and medication use diaries. If these were successfully completed and a nutritionist judged that the participant would be able to comply with the intervention protocol, an adaptive randomization procedure was used to assign participants to the intervention or control arms balancing on age, sex, and years from Barrett diagnosis. The intervention arm dietary goals were to reduce total dietary fat to 20% (or less) of total energy, increase fruit and vegetable servings to ≥6/d, and to avoid foods that participants associated with gastroesophageal reflux disease symptoms. Weight loss, a consequence of adopting a low-fat diet, was a secondary goal. The intervention was based on a program widely successful in women (19), which was modified to include domestic partners and more basic materials on nutrition and food preparation. The intervention was lead by a trained nutritionist and consisted of nine 1-hour individual sessions plus nine 90-minute group sessions. Participants randomized to the control arm of the study were asked to follow their usual diets.

**Biopsy Protocol.** Endoscopies were completed within 4 months before randomization and at 18 and 36 months after randomization, following published protocols (20-22). Briefly, using a large-channel endoscope, the gastroenterologist obtained four-quadrant biopsies for histology plus two biopsies for flow cytometry at 2-cm intervals throughout the columnar-lined epithelium, plus a biopsy from the gastric fundus. Specimens from the fundus and from each quadrant of each level of the tubular esophagus were examined by histology. Endoscopic landmarks, including the squamocolumnar junction, esophagogastric junction, and diaphragmatic impression, and extent of re-epithelialization (squamous islands) within the Barrett segment were recorded. Barrett esophagus segment length was defined as the distance between the esophagogastric junction and the squamocolumnar junction.

**Flow Cytometry.** The biopsies for flow cytometry were placed in tissue culture media with 5% FCS, 5 mmol/L HEPES buffer and 10% DMSO on ice during endoscopy and were stored at -70°C until analysis. Each biopsy was disassociated into a nuclear suspension and divided into two portions, one for use in single variable DNA content flow cytometry to detect the presence of aneuploidy and the percentage of cells in the 4N (G2/tetraploid) fraction of the cell cycle (16), and the other for Ki67/DNA content-dual variable flow cytometry to determine the percentage of Ki67-positive proliferating diploid G1 cells and the percentage of total Ki67-positive proliferating cells (22). It was necessary to divide the nuclear suspension from each biopsy, because centrifugation, which is required for the Ki67/DNA content-dual variable assay, introduces aggregates that may artifactually increase the 4N fraction assessed by single variable DNA content flow cytometry. Flow cytometry was done on a Coulter Elite ESP cytometer (Beckman-Coulter, Miami, FL); data were analyzed with MultiCycle and MultiPlus Software (Phoenix Flow Systems, San Diego, CA) and interpreted by the authors (P.S.R. and C.A.S.). Both the gastroenterologist and cytometrist were blinded to participant treatment arm.

**Diet and Anthropometric Assessments.** Diet was assessed with a 122-item self-administered, semiquantitative food frequency questionnaire at randomization and every 6 months after randomization. This food frequency questionnaire was designed to assess the dietary change goals of the intervention (23, 24). In addition, participants completed a telephone-administered 24-hour dietary recall in the week preceding the 18- and 36-month endoscopies. Recalls were unannounced (unscheduled) to minimize the effect of the dietary assessment on dietary behavior. The 24-hour dietary recall and the nutrient database for the food frequency questionnaire were based on the University of Minnesota Nutrient Data System (25).

At randomization, standardized procedures were used to collect height, weight, and waist and hip circumferences. Weight was assessed using the same procedures at 18 and 36 months after intervention.

**Symptom and Medication Diaries.** Seven-day symptom and medication use diaries were completed during the run-in period and during the week preceding the 18- and 36-month endoscopies. The symptom diary collected ratings for seven symptoms (day heartburn, night heartburn, abdominal pain, belching, regurgitation, nausea, and vomiting) and six complications (vomiting blood, bloody stool, aspiration, cough, difficulty swallowing, and painful swallowing). The medication diary collected type, daily frequency, and dose of all reflux medications, including antacids, H2 blockers, and proton pump inhibitors.

**Analysis.** All intervention effects were evaluated at 18 and 36 months after intervention. Effectiveness of the dietary intervention was evaluated as changes from baseline in total energy intake (kcal), percentage energy from fat (%en), daily servings (servings/d) of fruits and vegetables, and body weight. Dietary data were evaluated separately based on food frequency questionnaires and 24-hour recalls. The primary end points for evaluating the effect of the dietary intervention on Barrett esophagus were evaluated as (a) changes from baseline in the percentages of total cells and G1 cells positive for Ki67 (%G1-Ki67 and %Tot-Ki67, respectively), (b) the occurrence of ≥6% of G2/tetraploid cells (%4N), and (c) the occurrence of aneuploidy. Results for %G1-Ki67 and %Tot-Ki67 were calculated for the maximum and mean of all biopsies, which ranged from two biopsies for participants with short Barrett segments to 18 for those with long segments. A participant with aneuploid cell populations or %4N of ≥6 in any biopsy was considered positive for that outcome. Secondary end points were evaluated as change from baseline in the length and extent of squamous re-epithelialization of the Barrett segment. Re-epithelialization was scored 0 to 3 corresponding to “none,” “few,” “multiple,” or “extensive re-epithelialization.”
Intermediate or mediating effects of the intervention were evaluated as change from baseline in reflux symptoms and use of reflux medications. Based on a principal components factor analysis, symptom factors were defined as (a) swallowing problems (difficulty swallowing and painful swallowing), (b) gas (gas, belching, and abdominal pain), (c) heartburn (day heartburn and night heartburn), (d) nausea (nausea and vomiting), and (e) regurgitation (aspirations, regurgitation, and cough). Two rarely reported symptoms, vomiting blood and black/bloody stools, were excluded. Symptom factor scores were calculated for each day as the mean of each symptom score, coded 0 to 3 to correspond to ratings of "none," "mild," "moderate," or "severe." Prescription medication use was evaluated as the number of standard doses per day of H2 blockers and proton pump inhibitors using 300-mg ranitidine and 20-mg omeprazole, respectively, as the standard dose. For antacids, the standard dose was calculated as the 10-mEq acid-neutralizing capacity of one Tums. For both symptoms and medication use, analyses were based on the average from 7 days.

Multiple regression models were used to calculate intervention effects, defined as the difference in change from baseline to follow-up between intervention and control group participants. In these models, the dependent variable was change from baseline in the variable of interest, and independent variables included the baseline value of the variable of interest, sex, and a dummy variable indicating study arm. We control for baseline value to generate an estimate of change unbiased by baseline value and for sex, because of the relatively small number of women in the study. In this model, the regression coefficient for the study arm is the intervention effect adjusted for baseline value and demographic covariates. For %4N > 6 and aneuploidy, the study arm is the intervention effect adjusted for baseline value and demographic covariates for sex, because of the relatively small number of women in the study. In this model, the regression coefficient for the study arm is the intervention effect adjusted for baseline value and demographic covariates.

<table>
<thead>
<tr>
<th>Table 1. Baseline demographic characteristics, body mass index, smoking and alcohol use, by intervention arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
</tr>
<tr>
<td>Sex (% male)</td>
</tr>
<tr>
<td>Marital status (%)</td>
</tr>
<tr>
<td>Widowed/divorced/separated</td>
</tr>
<tr>
<td>Never married</td>
</tr>
<tr>
<td>Education (y)</td>
</tr>
<tr>
<td>Race/ethnicity (%)</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Household income (% US $1,000)</td>
</tr>
<tr>
<td>&lt;30</td>
</tr>
<tr>
<td>30-59</td>
</tr>
<tr>
<td>≥60</td>
</tr>
<tr>
<td>Current smoker (%)</td>
</tr>
<tr>
<td>Cigarettes, pipes and/or cigars</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>&lt;30</td>
</tr>
<tr>
<td>≥1 drinks/d (%)</td>
</tr>
</tbody>
</table>

Results

We randomized 93 patients with Barrett esophagus. Of these, four men in the control arm dropped out of the study immediately following randomization and are not included in subsequent analyses. One male control participant missed the 18-month but completed the 36-month biopsy and is thus included in the 36-month results only. After completing the 18-month biopsy, two participants died (one male in each treatment arm) and three (one female intervention, two male controls) dropped out of the study. Thus, there are 44 intervention and 44 control arm participants with at least some end point data at 18 months and 42 intervention and 42 control arm participants with end points at 36 months. At 18 months, one female intervention participant is missing flow cytometry results due to a damaged biopsy, and two males (one intervention and one control) did not complete a dietary assessment. The demographic and health-related characteristics of the study sample are given in Table 1. There were no statistically significant differences in characteristics between study arms. Participants were primarily overweight, White males, and only 10% were current smokers. Control participants were somewhat more likely to drink alcoholic beverages and, among those who did drink, report higher alcohol consumption.

Figure 1 shows the mean energy, fat, and fruit and vegetable consumption at each assessment. When estimated by food frequency questionnaires, at 18 months, intervention group participants decreased energy intake by 20% (−414 kcal) and percentage energy from fat by 34% (−10.7 percentage points) and increased fruit and vegetable intake by 41% (2 servings/d; all P < 0.001). At 36 months, changes from baseline remained statistically significant but were smaller for percentage energy from fat (−18%) and fruits and vegetables (35%). Among controls, dietary changes were modest and none reached statistical significance. Figure 1 also shows mean participant weight. Intervention group participants lost 3.6 kg by 18 months, which was reduced to 2.5 kg at 36 months (both P < 0.001); weight in control participants did not change. Table 2 gives intervention effects on diet and weight adjusted for baseline value, age, and sex. Intervention effects for percentage energy from fat and fruits and vegetables were −12.2 percentage points and 1.8 servings/d at 18 months, which decreased to −7.8 percentage points and 1.3 servings/d at 36 months. There was also a modest, nonsignificant intervention effect for reduced alcohol consumption among drinkers. Effects of the intervention based on 24-hour dietary recalls were as follows. Differences between intervention and control group participants for energy were −137 kcal at 18 months and −165 kcal at 36 months (both not significant). For percentage energy from fat, differences were −11.5 and −8.7 percentage points (both P < 0.001). Lastly, for alcohol differences, were −4.7 and −4.3 g/d (both not significant). The intervention effect for weight was −4.0 kg at 18 months (P < 0.005) and decreased to 1.4 kg (not significant) at 36 months. Overall, these results suggest that the intervention was successful in achieving goals for dietary change at 18 months, but there was moderate recidivism by 36 months.

The effects of the intervention on measures of cell proliferation in Barrett tissue are given in Table 3. There were no significant changes in either treatment arm in the mean or maximum %G1-Ki67 or %Tot-Ki67, with the exception of an increased mean %Tot-Ki67 at 18 months in the intervention arm. Intervention effects on cell proliferation measures were small and not statistically significant. The numbers of intervention and control participants developing %4N > 6 and corresponding relative risks with 95% confidence intervals at 18 months were 2 and 4 (relative risk, 0.5; 95% confidence interval, 0.1-2.6); and at 36 months, were 3 and 4 (relative risk, 0.75; 95% confidence interval, 0.2-3.1). When %4N was analyzed as a continuous variable, there were small, statistically nonsignificant positive intervention effects;
however, a biological interpretation of changes in %4N below the 6% cut point are not well motivated. There was a statistically significant decrease of 0.5 cm in Barrett segment length among participants in the treatment arm, but at no time point did the intervention effect on segment length reach statistical significance. Finally, only one participant (control) developed aneuploidy; thus, this outcome was too rare to evaluate statistically.

Table 4 gives results for medication use and symptoms. There were no significant intervention effects on use of prescription medications (H2 blockers plus proton pump inhibitors) or use of antacids. Additional analyses also found no intervention effect evaluating H2 blockers and proton pump inhibitors alone (data not shown). There was also little effect of the intervention on symptoms. Symptom scores were generally low throughout the study, and there were no significant changes in symptoms in either treatment arm. However, there was a significant treatment effect for reduced heartburn at 36 months.

Exploratory analyses, examining treatment outcomes stratified by sex and age, were consistent with results for the study sample overall.

Discussion

We found no evidence that a dietary intervention to reduce fat intake and increase consumption of fruits and vegetables affected proliferation-associated biomarkers in Barrett esophagus over a 3-year period. Nor did we find evidence of effectiveness of the dietary intervention in subgroup analyses stratified by sex and age. The dietary intervention did result in significant weight loss, but modification of this well-established risk factor did not affect proliferative biomarkers.

These results were unexpected, given the consistency of studies that find strong associations of obesity and fat intake with increased risks, and fruit and vegetable consumption with decreased risks of esophageal adenocarcinoma. Multiple case-control studies have found that low intakes of dietary fiber (9, 26-30) and fruits and vegetables (26, 29, 31-33), high intakes of fat (9, 27-30), and obesity (10, 12, 17, 26, 32, 34-36) are risk factors for adenocarcinoma of the esophagus and gastric cardia. The literature on the associations of diet and obesity with gastroesophageal reflux disease is similar but less consistent. Whereas obesity is associated with gastroesophageal reflux disease in multiple studies (37-42), the association between dietary fat and gastroesophageal reflux disease is controversial. In rat models, dietary fat in conjunction with exposure of the distal esophagus to gastroduodenal juice promotes development of Barrett esophagus and esophageal adenocarcinoma (43, 44). Experimental studies in humans have generally failed to find an association between dietary fat and acid reflux. Studies comparing equicaloric meals of differing fat content in healthy subjects and in patients with gastroesophageal reflux disease have found that there was no

Table 2. Intervention effects on percentage of energy from fat, energy, fruits and vegetables, alcohol and weight at 18 and 36 months.

<table>
<thead>
<tr>
<th>Time from randomization (mo)</th>
<th>Fat (% en)*</th>
<th>Energy (kcal)</th>
<th>Fruits and vegetables (servings/d)</th>
<th>Alcohol (g)</th>
<th>Weight (lbs)</th>
<th>Intervention (n)</th>
<th>Control (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>–12.2 ± 1.4*</td>
<td>–314 ± 107*</td>
<td>1.8 ± 0.4*</td>
<td>–2.4 ± 1.5</td>
<td>–4.0 ± 1.1</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>36</td>
<td>–7.8 ± 1.4*</td>
<td>–370 ± 106*</td>
<td>1.3 ± 0.4*</td>
<td>–2.1 ± 1.8</td>
<td>–1.4 ± 1.2</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

NOTE: Mean ± SE, adjusted for baseline value and sex.

*Percentage of energy.

*P < 0.0001.

*P < 0.005.
### Table 3. Effects of dietary intervention on proliferation-associated biomarkers in Barrett esophagus

<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SD) Int = 44, Con = 45</th>
<th>18 mo (mean change ± SE) Int = 43, Con = 44</th>
<th>36 mo (mean change ± SE) Int = 42, Con = 42</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G1-Ki67+ (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean†</td>
<td>20.1 ± 6.5</td>
<td>2.5 ± 1.5</td>
<td>1.7 ± 1.6</td>
</tr>
<tr>
<td>Intervention effect†</td>
<td>0.96 ± 1.58</td>
<td>1.79 ± 1.31</td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>28.7 ± 8.4</td>
<td>1.7 ± 1.7</td>
<td>0.5 ± 1.9</td>
</tr>
<tr>
<td>Intervention effect</td>
<td>−0.01 ± 1.96</td>
<td></td>
<td>1.01 ± 2.08</td>
</tr>
<tr>
<td>Total Ki67† (%)</td>
<td>32.7 ± 8.5</td>
<td>2.1 ± 1.8</td>
<td>1.0 ± 1.8</td>
</tr>
<tr>
<td>Mean</td>
<td>6.0 ± 3.1</td>
<td>0.0 ± 0.1</td>
<td>−0.5 ± 0.1†</td>
</tr>
<tr>
<td>Intervention effect</td>
<td>−0.04 ± 0.25</td>
<td></td>
<td>−0.37 ± 0.28</td>
</tr>
</tbody>
</table>

*Cells in G1 staining positive for Ki67.
†Mean of between 2 and 18 biopsies, taken in pairs at 2-cm intervals.
‡Mean ± SE, change in intervention minus change in control, adjusted for baseline and sex.
§Total cells staining positive for Ki67.
*P < 0.05.
*P < 0.001.

A difference in esophageal acid exposure or mean number of reflux episodes in response to a high-fat versus low-fat meal (45-48). However, one study also examined effects of total energy as well as fat content of meals and found that a high-energy meal with the same percentage of calories from fat as a low-energy meal increased esophageal acid exposure and mean number of reflux episodes (48). Thus, the weight loss target of this dietary intervention seems well motivated, but

### Table 4. Effects of dietary intervention on medication use and gastroesophageal reflux symptoms

<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SD) Int = 44, Con = 45</th>
<th>18 mo (mean change ± SE) Int = 43, Con = 44</th>
<th>36 mo (mean change ± SE) Int = 42, Con = 42</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Medications</em> (dose/d)</em>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int</td>
<td>0.94 ± 0.39</td>
<td>0.03 ± 0.04</td>
<td>0.03 ± 0.07</td>
</tr>
<tr>
<td>Con</td>
<td>0.91 ± 0.51</td>
<td>−0.02 ± 0.07</td>
<td>−0.00 ± 0.09</td>
</tr>
<tr>
<td>Intervention effect†</td>
<td>0.05 ± 0.09</td>
<td></td>
<td>−0.01 ± 0.11</td>
</tr>
<tr>
<td><strong>Antacids (dose/d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int</td>
<td>0.16 ± 0.40</td>
<td>0.06 ± 0.12</td>
<td>0.05 ± 0.06</td>
</tr>
<tr>
<td>Con</td>
<td>0.47 ± 1.27</td>
<td>−0.01 ± 0.15</td>
<td>−0.03 ± 0.25</td>
</tr>
<tr>
<td>Intervention effect</td>
<td>1.00 ± 1.68</td>
<td></td>
<td>1.80 ± 1.45</td>
</tr>
<tr>
<td><strong>Symptoms†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>0.01 ± 0.04</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Swallowing</td>
<td>0.07 ± 0.23</td>
<td>0.06 ± 0.03</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>C guardian</td>
<td>0.21 ± 0.35</td>
<td>0.07 ± 0.04</td>
<td>0.01 ± 0.04</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.14 ± 0.23</td>
<td>−0.05 ± 0.04</td>
<td>−0.04 ± 0.04</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>0.11 ± 0.21</td>
<td>0.01 ± 0.03</td>
<td>0.04 ± 0.06</td>
</tr>
<tr>
<td>Intervention effect</td>
<td>0.16 ± 0.23</td>
<td>−0.03 ± 0.04</td>
<td>−0.04 ± 0.03</td>
</tr>
</tbody>
</table>

*H2 receptor antagonists and proton pump inhibitors.
†Mean ± SE, change in intervention minus change in control, adjusted for baseline and sex.
‡See Materials and Methods for scale score interpretation.
*P < 0.05.
whether any effect of dietary fat reduction would be direct or due to the fact that low-fat diets lead to weight loss, is unclear. One possibility for the lack of an effect of dietary change could be poor adherence to the behavioral intervention. We used a dietary intervention protocol that, with minor modification, was based on previous successful randomized trials (19, 49, 50). There was good evidence that the intervention was effective in changing dietary behavior, and the magnitude of these changes were similar to those seen in previous studies: percentage energy from fat was reduced by 33% and intervention group participants lost 3.2 kg compared with a 0.5 kg weight gain among control participants. It remains possible, however, that dietary changes were not of sufficient magnitude or duration to observe changes in these proliferative biomarkers of Barrett esophagus.

Another reason for a negative outcome could be that the outcome measures were not sensitive to the intervention. It is possible that the intervention could affect earlier stages of neoplasia (e.g., shortening of telomere length, oxidative DNA damage, or development of Barrett esophagus). As >90% of all Barrett segments have an abnormality in at least one copy of the p16 gene and these abnormalities can involve the entire Barrett segment (51), it may be that hyperplasia, as measured by Ki67, is a property of the genetically abnormal Barrett metaplasia that cannot be reversed with dietary intervention. In addition, based on our most recent work, the strongest predictors of adenocarcinoma among persons with Barrett esophagus are clonal genetic biomarkers, including aneuploidy, accumulation of ≥6% cells in the G2/M fraction of the cell cycle, and 17p (p53) loss of heterozygosity (16, 52). These outcomes were too rare in this study to evaluate whether they would be affected by dietary intervention.

We conclude that a short-term intervention to decrease dietary fat intake, increase consumption of fruits and vegetables, and promote weight loss has no effect on proliferation-associated flow cytometric biomarkers of neo-plastic progression or clinical observations in Barrett esophagus. Whether an intervention based on other factors associated with markers of progression of Barrett esophagus, including selenium supplementation or nonsteroidal anti-inflammatory agents, would be effective, remains to be tested.

References
Low-Fat, High Fruit and Vegetable Diets and Weight Loss Do Not Affect Biomarkers of Cellular Proliferation in Barrett Esophagus


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