Vitamin C Intake and Apoptosis in Normal Rectal Epithelium

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Introduction

Apoptosis, or programmed cell death, may lower the risk of neoplasia by removing genetically damaged or mutated cells. A high rate of apoptosis has been linked to a reduced risk of colorectal adenomas; therefore, it is important to understand factors that impact apoptosis. Antioxidants (e.g., vitamin C) protect cells from harmful oxidation processes but may interfere with apoptosis by protecting genetically damaged cells from reactive oxygen species-dependent cell death. The objective of this study was to evaluate the association between vitamin C intake and apoptosis in normal rectal mucosa. Study participants were part of a large, cross-sectional study, the Diet and Health Study III. Participants were recruited from consecutive, consenting patients who underwent colonoscopy at University of North Carolina Hospitals between August 1, 1998 and March 4, 2000. Vitamin C intake, obtained from a food frequency questionnaire, included both dietary sources and vitamin supplements. Apoptosis was measured by morphological evaluation of H&E-stained sections obtained from pinch biopsy samples of normal rectal mucosa in consenting participants (n = 503). The relationship between vitamin C and apoptosis varied by adenoma status. Among individuals with adenomas, there was an inverse linear association between apoptosis and total vitamin C intake. Similarly, individuals with adenomas in the highest quintile of total vitamin C intake were substantially less likely than those in the lowest quintile to have increased colonic apoptosis (odds ratio, 0.05; 95% confidence interval, 0.01–0.46). Vitamin C was not significantly associated with apoptosis in adenoma-free patients. High vitamin C intake was associated with reduced colorectal apoptosis among individuals with adenomas in this study population. Given that high apoptosis may lower colorectal cancer risk, vitamin C supplements may be contraindicated for patients with a history of adenomas.

Abstract

Apoptosis, or programmed cell death, may lower the risk of neoplasia by removing genetically damaged or mutated cells. A high rate of apoptosis has been linked to a reduced risk of colorectal adenomas; therefore, it is important to understand factors that impact apoptosis. Antioxidants (e.g., vitamin C) protect cells from harmful oxidation processes but may interfere with apoptosis by protecting genetically damaged cells from reactive oxygen species-dependent cell death. The objective of this study was to evaluate the association between vitamin C intake and apoptosis in normal rectal mucosa. Study participants were part of a large, cross-sectional study, the Diet and Health Study III. Participants were recruited from consecutive, consenting patients who underwent colonoscopy at University of North Carolina Hospitals between August 1, 1998 and March 4, 2000. Vitamin C intake, obtained from a food frequency questionnaire, included both dietary sources and vitamin supplements. Apoptosis was measured by morphological evaluation of H&E-stained sections obtained from pinch biopsy samples of normal rectal mucosa in consenting participants (n = 503). The relationship between vitamin C and apoptosis varied by adenoma status. Among individuals with adenomas, there was an inverse linear association between apoptosis and total vitamin C intake. Similarly, individuals with adenomas in the highest quintile of total vitamin C intake were substantially less likely than those in the lowest quintile to have increased colonic apoptosis (odds ratio, 0.05; 95% confidence interval, 0.01–0.46). Vitamin C was not significantly associated with apoptosis in adenoma-free patients. High vitamin C intake was associated with reduced colorectal apoptosis among individuals with adenomas in this study population. Given that high apoptosis may lower colorectal cancer risk, vitamin C supplements may be contraindicated for patients with a history of adenomas.

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The abbreviations used are: ROS, reactive oxygen species; OR, odds ratio; CI, confidence interval; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; DHS III, Diet and Health Study III; UNCH, University of North Carolina Hospital; FFQ, food frequency questionnaire; BMI, body mass index.
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The null or negative results from antioxidant chemoprevention trials. Although free radicals (cellular oxidants, a subset of ROS) are usually described as harmful to cellular systems, they are also critical mediators of antimicrobial phagocytosis, detoxification reactions, and apoptosis (8). Because apoptosis is a pathway by which precancerous and other potentially dangerous cells are eliminated, antioxidants could interfere with the natural elimination of harmful or unhealthy cells by preventing ROS-dependent apoptosis. Therefore, high antioxidant levels might actually reduce anticancer apoptotic activity, particularly in persons with low levels of endogenous ROS (7, 8, 16–19). The notion that antioxidants may interfere with the apoptotic process may explain, at least in part, why recent chemoprevention trials have yielded surprisingly null results.

The purpose of the present analysis was to investigate the association between vitamin C, a potent antioxidant, and apoptosis. Specifically, we undertook an analysis to examine whether high intake of vitamin C is associated with reduced apoptosis in normal colonic mucosa.

Materials and Methods

Study Population. The study population was the DHS III, a large, cross-sectional study conducted at the UNCH (Chapel Hill, NC). UNCH is a 700-bed university hospital serving as the primary health care facility for residents of Orange and adjacent counties and as a referral center for much of the state. Patients are diverse with respect to socioeconomic status and race/ethnicity.

The DHS III Study was designed to investigate dietary and lifestyle factors associated with colorectal adenomas, generally believed to be precursors of colorectal adenocarcinomas. Study participants were recruited from consecutive, consenting patients who underwent colonoscopy between August 1, 1998 and March 4, 2000. Eligibility requirements included: age ≥ 30 years; ability to understand and complete an interview in English; and no evidence of familial polyposis, colitis, previous colonic resection, or previous colon cancer or adenoma. Patients who did not satisfactorily prepare the colon for the procedure were excluded, as were those with incomplete visualization from the colon to the cecum. Eligible patients were asked to allow the colonoscopist to obtain rectal biopsy specimens from normal mucosa, in addition to subsequently completing a telephone-administered interview.

Data Collection. During the enrollment period, 2452 out-patient colonoscopies were performed at UNCH; 1526 potential participants were excluded based on eligibility or an unsatisfactory procedure. Reasons for exclusion were not mutually exclusive and included previous adenomas (n = 615), colitis (n = 405), previous colon resection (n = 311), younger than age 30 years (n = 267), previous colon cancer (n = 220), incomplete examination (n = 182), inability to give informed consent (n = 156), unsatisfactory preparation (n = 149), polypsis (n = 6), and other factors (n = 73). Among the 926 remaining eligible participants, 57 (6.2%) refused, and 66 (7.1%) were not asked because the research assistant was unavailable, leaving 803 (93.4%) who consented to participate in the study. Of the 803 consenting patients, 503 also agreed to the rectal pinch biopsies. Therefore, the final sample size for this analysis was 503.

All biopsies were obtained 8–10 cm from the anal verge using standard (8-mm wing) disposable, fenestrated colonoscopy forceps with a central spike (Wilson-Cook, Winston-Salem, NC), yielding a specimen 3–4 mm in diameter. Biopsies were taken from normal-appearing mucosa, avoiding raised lesions or larger blood vessels.

Apoptosis was measured from biopsy specimens in two ways: (a) strict morphological criteria (examination of H&E-stained sections under light microscopy); and (b) TUNEL staining. In the first method, two biopsies were placed in 10% buffered formalin for routine histology and prepared with H&E stain. Each prepared slide had five levels of tissue sectioned at least 50 μm apart, from which 8–12 well-oriented colonic crypts were scored (per biopsy). Apoptotic cells were identified by chromatin condensation at the nuclear boundary, membrane blebbing, and cytoplasmic shrinkage. An experienced technician, blinded to adenoma status, scored all sections. The scoring technique had a reproducibility of 99%. Apoptosis was scored as the number of apoptotic cells/colonic crypt. For each patient, the mean number of apoptotic cells was derived by taking the average number of apoptotic cells from the two biopsy specimens (i.e., from a total of 16–24 well-oriented crypts). A second method, TUNEL staining, was also used; however, recent studies have cited problems with this assay (20, 21). Prolonged tissue fixation, tissue handling, increased nonspecific staining, need to optimize (to avoid over- and understaining), and specificity are some of the problems that may interfere with the TUNEL assay and lead to inaccurate determination of apoptosis. For these reasons, we feel that morphological identification of apoptosis is a better reflection of apoptosis than TUNEL staining, and therefore we used the morphological measure of apoptosis in the present analysis. We also note that the relationship between vitamin C and apoptosis was essentially the same when apoptosis was measured with TUNEL as it was when measured using histological measures.

Dietary information (i.e., dietary vitamin C and total energy intakes) was obtained using a modified version of the Block-NCI FFQ (22). The version of the Block quantitative FFQ used in this study was specially modified for use in North Carolina to include regional foods (23). The FFQ was administered by telephone within 12 weeks of colonoscopy, and the reference period for dietary intake was in the past year, to account for seasonal variations in diet. We elected to examine vitamin C rather than other antioxidants (e.g., vitamin E or selenium) because intake of vitamin E from foods is relatively small, and selenium consumption may not be well captured by FFQs (24).

Supplement use was assessed with closed-ended questions about the use of multiple vitamins (e.g., Centrum and other antioxidant combinations) as well as single vitamin C supplements. We inquired, specifically, about duration (in years), frequency (days/week), and usual dose (e.g., 100, 250, 500, 750, or 1000 mg) of vitamin C supplements over the previous year. Dietary vitamin C intake, calculated by the BLOCK analysis program, was combined with supplemental intakes to obtain total vitamin C intake.

Statistical Analysis. Univariate analysis was used to examine the distributions, delete outliers, and identify implausible values of apoptosis and vitamin C intake. The linear relationship between vitamin C and apoptosis was investigated using multiple linear regression analysis. Candidate confounders for potential inclusion in models were identified by comparison of means of vitamin C intake and apoptosis between levels of each potential confounder (age, sex, race, nonsteroidal anti-inflammatory drug use, smoking, total energy, and BMI) in bivariate analyses. If a statistically significant difference in mean vitamin C intake and apoptosis across levels of the confounder was observed, that variable was further assessed, using a change in
estimate approach, in subsequent modeling. We also evaluated effect measure modification by presence or absence of adenoma. Multiple linear regression modeling was conducted using a backward elimination strategy. Apoptosis and total vitamin C intake were log-transformed to fulfill the normality assumption of the linear regression model.

Stratified and multiple logistic regression analyses were conducted to investigate the relationship between vitamin C intake and apoptosis. Logistic regression modeling was done by backwards elimination. Potential confounders identified from bivariate and stratified analysis were assessed as confounders of the multivariate model using a change in main effect estimate of 20%. The log likelihood test was used to identify effect modifiers. Logistic regression was used to model the odds of high apoptosis (higher than the study population median value for apoptosis) in comparison with low apoptosis. Exposure effects were separately assessed for dietary and for total vitamin C intake in quintiles derived from the distribution of all participants in our study sample. The standard multivariate technique was used to adjust for total energy intake (25).

All analyses were conducted in SAS, version 8.1 (SAS Institute, Cary, NC).

Results

The mean age of participants was 56 years (SD = 11 years), 36% were 60 years or older, 40% were male, almost three-quarters were white, and 31% were obese (BMI \( \geq 30 \) kg/m\(^2\)). One hundred and seventy participants (35%) had at least one colorectal adenoma. The mean total vitamin C intake was 313 mg/day (range, 20–1589 mg/day), and the mean dietary vitamin C intake was 191 mg/day (range, 19–656 mg/day). The median score for apoptotic cells/crypt was 2.74 (range, 1.3–5.3). There is no standard for high or low apoptosis in human tissue; therefore, high or low apoptosis was defined as above or below this study sample median.

Table 1 gives mean total and dietary vitamin C intake and mean number of apoptotic cells/crypt, stratified by demographic and health-related characteristics. There was a statistically significant difference in mean apoptosis between categories of total energy intake by adenoma status. Specifically, respondents who consumed fewer than 1500 kcal/day (excluding alcohol) had lower apoptosis than those consuming more than 1500 kcal/day. Participants with one or more adenomas had lower rates of apoptosis than those without adenomas. In addition, obese, overweight, and normal weight participants had substantially lower rates of apoptosis than underweight participants, all \( P \leq 0.05 \).

Mean total vitamin C intake also differed by certain participant characteristics. African Americans had statistically significantly lower mean daily vitamin C intakes than whites; men consumed less total vitamin C than women; and current smokers reported lower intakes than former or never smokers. The most striking difference was for BMI: participants with normal BMI had significantly higher mean total vitamin C intakes (368 mg) in comparison with underweight or obese participants (194 and 251 mg, respectively). Respondents who consumed fewer than 1000 kcal/day (excluding alcohol) also had lower intakes of total vitamin C (208 mg) than those consuming more than 1000 kcal/day (338 mg).

Neither total nor dietary vitamin C intake was correlated with apoptosis in the crude (unadjusted) analysis (Pearson’s \( r = 0.03 \) and \( r = -0.01 \), respectively). Bivariate analyses revealed a U-shaped relation between quintiles of total vitamin C and apoptotic activity; however, this association was not statistically significant (\( P = 0.55 \)).

Total energy intake was the only confounder in both the multiple linear and logistic regression models. After adjustment for total energy, there was a decrease in apoptosis with increasing total vitamin C intake, but only among participants with adenomas (\( \beta = -0.0443; P = 0.06 \); Fig. 1). Among individuals without adenomas, there was no significant linear association between total vitamin C intake and apoptosis (\( \beta = 0.0059; P = 0.70 \)). Similar results were observed for dietary vitamin C (adenoma, \( \beta = -0.0570 \) and \( P = 0.08 \); adenoma free, \( \beta = -0.0023 \) and \( P = 0.92 \)).

Table 2 shows the results from multivariate logistic regression analysis, adjusted for total energy intake. As in the multiple linear regression analysis, the association between vitamin C and apoptosis was confounded by energy intake and modified by adenoma status. (Unadjusted results were in the same direction but of lesser magnitude than energy-adjusted results.) Among participants with adenomas, odds of high apoptosis decreased with increasing quintiles of total vitamin C intake. The odds of high apoptosis were significantly lower in the highest versus the lowest quintile of total vitamin C intake (OR for the highest quintile, 0.05; 95% CI, 0.01–0.46). Although most ORs were not statistically significant, higher total vitamin C intake was associated with lower odds of high apoptosis in both adenoma and adenoma-free participants. Patterns in dietary vitamin C intake were less clear.

Discussion

In this study of 503 men and women in North Carolina, we found that total vitamin C intake was inversely associated with apoptosis among participants with adenomas. Previous research has suggested an association between inhibition of apoptosis and colorectal cancer development (26, 27). Additionally, in a previous analysis of this study population, low apoptosis was associated with a 7-fold increased odds for the presence of colorectal adenomas (28). This result implies that apoptosis may be a very strong predictor of adenoma development, a precursor lesion of colorectal cancer. Given this finding, it is important to identify modifiable factors that influence human apoptotic activity to develop risk reduction strategies for colon cancer and possibly other cancers. Furthermore, many chemotherapy treatments work through apoptotic pathways, thus our results could also be relevant to cancer treatment.

In light of our findings, the null results observed in three published antioxidant polyp prevention trials (13–15) are not surprising. Specifically, our results suggest that persons who tend to develop adenomas may be more susceptible to a reduction in apoptosis following high antioxidant consumption. One explanation for this effect could be lower levels of endogenous ROS in persons prone to developing adenomas (8). If this is the case, high antioxidant levels might actually reduce anticancer apoptotic activity (i.e., ROS-dependent apoptosis) because of a high ratio of antioxidants to oxidants in these individuals.

Because we had no measurements of endogenous ROS levels, we could not investigate low ROS status as a possible explanation for the stronger inverse association between vitamin C and apoptosis in those with adenomas. It is possible that those at high risk of eliminating too many free radicals (because of lower endogenous ROS levels) are at increased risk for cancer at high levels of antioxidant consumption. This pathway warrants future investigation.

We measured apoptosis in normal rectal mucosa, rather than in the adenomatous tissue. The primary reason for this was...
to address one of the objectives of the DHS III Study: to compare apoptosis in those with and without adenomas. Apoptosis was measured in tissue obtained from the same rectal location in all participants, irrespective of whether or not they had an adenoma. Previously, we found that low apoptosis was strongly related to the presence of adenoma(s) in the colon (28). This suggests a “field effect” in the colon because low apoptosis was associated with the presence of adenomas located anywhere in the colon. Thus, a low rate of apoptosis may indicate a higher risk for developing adenomas.

Findings from our previous work suggest that low apoptosis in normal rectal mucosa is a physiologically inappropri-

### Table 1  Mean apoptosis and total vitamin C intake by demographic and health-related characteristics of the DHS III population (n = 503)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Mean no. of apoptotic cells/crypt</th>
<th>Mean total vitamin C intake (mg/day)a</th>
<th>Mean dietary vitamin C intake (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>74 (14.9)</td>
<td>2.86</td>
<td>269</td>
<td>199</td>
</tr>
<tr>
<td>45–59</td>
<td>242 (48.8)</td>
<td>2.75</td>
<td>334</td>
<td>192</td>
</tr>
<tr>
<td>≥60</td>
<td>180 (36.3)</td>
<td>2.72</td>
<td>306</td>
<td>179</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>119 (23.8)</td>
<td>2.79</td>
<td>210</td>
<td>173</td>
</tr>
<tr>
<td>White</td>
<td>364 (72.7)</td>
<td>2.75</td>
<td>346</td>
<td>197</td>
</tr>
<tr>
<td>Other</td>
<td>18 (3.6)</td>
<td>2.78</td>
<td>332</td>
<td>186</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>202 (40.2)</td>
<td>2.70</td>
<td>281c</td>
<td>201</td>
</tr>
<tr>
<td>Female</td>
<td>301 (59.8)</td>
<td>2.80</td>
<td>335</td>
<td>184</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>245 (48.8)</td>
<td>2.76</td>
<td>330</td>
<td>193</td>
</tr>
<tr>
<td>Former smoker</td>
<td>167 (33.3)</td>
<td>2.70</td>
<td>341</td>
<td>208</td>
</tr>
<tr>
<td>Current smoker</td>
<td>90 (17.9)</td>
<td>2.88</td>
<td>215c</td>
<td>143c</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5 (underweight)</td>
<td>13 (2.6)</td>
<td>3.19c</td>
<td>194c</td>
<td>185</td>
</tr>
<tr>
<td>18.5–24.9 (normal)</td>
<td>165 (33.3)</td>
<td>2.80</td>
<td>368c</td>
<td>199</td>
</tr>
<tr>
<td>25.0–29.9 (overweight)</td>
<td>160 (32.3)</td>
<td>2.76</td>
<td>330</td>
<td>194</td>
</tr>
<tr>
<td>≥30.0 (obese/morbidly obese)</td>
<td>158 (31.9)</td>
<td>2.70</td>
<td>251</td>
<td>178</td>
</tr>
<tr>
<td><strong>Total energy intake (kcal/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>107 (21.3)</td>
<td>2.69</td>
<td>208c</td>
<td>114c</td>
</tr>
<tr>
<td>1000–1500</td>
<td>168 (33.4)</td>
<td>2.67</td>
<td>331</td>
<td>179c</td>
</tr>
<tr>
<td>1501–2000</td>
<td>131 (26.0)</td>
<td>2.85</td>
<td>361</td>
<td>224</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>97 (19.3)</td>
<td>2.84</td>
<td>332</td>
<td>252</td>
</tr>
<tr>
<td>**NSAID use (during past 5 yrs)**d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasional/never</td>
<td>224 (45.2)</td>
<td>2.81</td>
<td>314</td>
<td>191</td>
</tr>
<tr>
<td>Regular user</td>
<td>272 (54.8)</td>
<td>2.72</td>
<td>313</td>
<td>190</td>
</tr>
<tr>
<td><strong>Adenoma status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one adenoma</td>
<td>173 (35.0)</td>
<td>2.46c</td>
<td>290</td>
<td>193</td>
</tr>
<tr>
<td>No adenomas</td>
<td>321 (65.0)</td>
<td>2.93</td>
<td>329</td>
<td>185</td>
</tr>
<tr>
<td><strong>Colonoscopy preparation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golytely</td>
<td>158 (31.6)</td>
<td>2.83</td>
<td>314</td>
<td>194</td>
</tr>
<tr>
<td>Phosphosoda</td>
<td>342 (68.4)</td>
<td>2.73</td>
<td>313</td>
<td>189</td>
</tr>
</tbody>
</table>

a Vitamin C from dietary plus supplemental sources.
b P for the overall F-statistic.
c Statistically significant difference in means at the P < 0.05 level between this category and all other categories for this characteristic with Bonferroni correction for multiple comparisons.
d NSAID (nonsteroidal anti-inflammatory): regular use ≥ 3 or more times/week, occasional use < 3 times/week.
Notes: numbers do not sum to 503 for all characteristics due to missing data; data in this table are unadjusted for other covariates.
ate state and disadvantageous with regard to adenoma risk (28). On the other hand, it is possible that in some individuals, reduced apoptosis in normal rectal tissue is physiologically appropriate (i.e., an indication of a low stress environment receiving low rates of DNA hits). We have no means of identifying a low stress environment in the current study; however, this hypothesis could be evaluated in future studies.

It is also conceivable that antioxidants repair damage caused by ROS, thereby reducing the number of cells that need to be eliminated by the apoptotic mechanism. Low apoptosis would not be considered an undesirable condition, in this scenario, because it results from a reduced need for cell elimination. However, in terms of adenoma risk, low apoptosis in normal rectal mucosa appears to be disadvantageous (28).

Bivariate analyses (Table 1) show an unexpected relationship between apoptosis and energy consumption. Some studies have suggested that low energy intake is associated with a reduced risk of cancer (29–31). In our study, participants with lower total energy intake, in which one would expect a reduced risk for adenoma, also had lower apoptosis levels. On the other hand, as one would expect, those with very low BMI had higher rates of apoptosis, whereas those with high BMI had lower rates of apoptosis. Because overweight individuals tend to underreport energy intake (32), it is conceivable that there was misclassification of energy intake among those with low rates of apoptosis (i.e., high-BMI individuals).

Results of studies examining associations of antioxidants with apoptosis are mixed. Some experimental studies have found that antioxidants reduce apoptosis (19, 33–36), whereas others have not (37, 38). Very few published studies have specifically examined the association between vitamin C intake and apoptosis in humans; however, in a study of patients with congestive heart failure, Rossig et al. (39) found that the administration of vitamin C significantly reduced apoptosis.

It has been proposed that the cancer-protective effect of vegetable and fruit consumption is partly due to antioxidant properties that allow scavenging of free radicals that, in turn, prevent DNA damage and subsequent mutation (40). Several randomized clinical trials of β-carotene and other antioxidant supplements have attempted to identify the specific components of fruits and vegetables that might cause a reduction in cancer risk. Five well-known trials showed that these vitamin supplements did not reduce the incidence of adenomas and other cancers (11–15); in fact, in the β-Carotene and Retinol Efficacy Trial, β-carotene supplements increased lung cancer risk in smokers (12). It is important to note that dietary vitamin C may have a different effect than supplemental vitamin C because of other components in the fruits and vegetables containing the vitamin (41). In our analyses, results for total and dietary vitamin C were generally similar; however, total and dietary vitamin C were only modestly correlated in our population (r = 0.45; P < 0.01).

Our findings have potential implications for cancer patients because many radiation and chemotherapeutic agents work by inducing apoptosis (5, 42, 43). If vitamin C protects cancer cells from ROS-dependent apoptosis, high vitamin C intake during treatment could potentially reduce the efficacy of chemotherapy treatments. There is evidence, however, that not all apoptosis involves ROS and not all chemotherapy agents enhance apoptosis by ROS-dependent mechanisms (44). Also, vitamin C may provide health benefits by mechanisms distinct from its antioxidant properties. Vitamin C may, for example, modify the metabolism of polycyclic hydrocarbons, leading to reduced mutagenic activity (45). Therefore, even if vitamin C reduces therapy-induced apoptosis of tumor cells through prevention of oxidation, the efficacy of all chemotherapies might not be influenced by high vitamin C intake. Our findings suggest that interactions between vitamin C and therapeutic agents are worthy of further investigation.

There are several features that make our study notable. Most importantly, this is one of very few studies that have examined apoptosis in human volunteers, and we were able to evaluate the distribution of apoptosis across various demographic and behavioral characteristics. Most studies of apoptosis have used animal or cell models. We also had a large sample size for this type of biological data, in conjunction with carefully collected diet and lifestyle information.

Certain limitations must be acknowledged. First, estimates of vitamin C from FFQs are not as precise as would be ideal; biological measures may be more desirable. However, validation studies have reported modest correlations between vitamin C intakes derived from FFQs and plasma or leukocyte vitamin
C [r = 0.43 and r = 0.31, respectively (46)], and several other studies have used vitamin C estimates from FFQs to study diet and cancer/adenoma associations (47–50). In addition, a recent study found that plasma ascorbic acid provided a similar ranking of individual intake compared with FFQ-derived vitamin C intake (51).

Second, the relationship of vitamin C with apoptosis may be modified by other antioxidants such as vitamin E and selenium status, but a larger study population or better assessment of these nutrients is needed to adequately explore these pathways. Third, we have no knowledge of daily, weekly, or monthly variation of apoptotic rates in humans, nor do we know how sensitive apoptosis is to changes in diet or other health-related factors. Also, recent changes in vitamin C consumption, which could have a direct effect on apoptosis, could not be evaluated. In future studies, it would be useful to obtain repeated measures of both vitamin C and apoptosis to assess longitudinal patterns of apoptosis in response to environmental changes.

In conclusion, vitamin C intake was inversely associated with apoptosis among participants with adenomas. Because high apoptosis has been shown to be strongly associated with a reduced presence of adenomas, vitamin C supplementation may negatively impact the pathway of colorectal neoplasia, particularly among persons with adenomas. It also appears that there may be a different mechanism of antioxidant action for certain subgroups of the population. Because of conflicting results regarding the benefits of antioxidants on chronic disease initiation or progression, persons at higher risk of adenomas should be encouraged to maintain a healthy diet, including regular consumption of fruits and vegetables (52). Our results suggest, however, that in terms of colorectal cancer risk, consumption of excessive vitamin C supplements may be contraindicated among individuals with a history of adenomas.

References


