Carotenoids in human buccal mucosa cells after 4 wk of supplementation with tomato juice or lycopene supplements

Inke Paetau, David Rao, Eugene R Wiley, Ellen D Brown, and Beverly A Clevidence

ABSTRACT

Background: Lycopene has been identified as a phytochemical with potentially protective health benefits.

Objective: Our objective was to monitor lycopene changes in buccal mucosa cells (BMCs) in response to 3 vehicles for oral delivery of lycopene.

Design: Fifteen healthy subjects ingested lycopene-rich tomato juice, tomato oleoresin, lycopene beadlets (each containing 70–75 mg lycopene) and a placebo for 4 wk each in a randomized crossover design while consuming self-selected diets. A 6-wk washout period separated the treatment periods. BMCs were collected at baseline and after 4 wk of supplementation.

Results: Lycopene in BMCs increased significantly (≈2-fold) after 4 wk of ingestion of oleoresin and of beadlets to 4.95 (P < 0.001) and 3.75 μg/g protein (P = 0.053), respectively, but was not significantly affected by tomato juice treatment. The placebo treatment produced a significant decrease in BMC lycopene concentrations (P = 0.018). We observed significant treatment differences between oleoresin and tomato juice, oleoresin and placebo, and beadlets and placebo. BMC concentrations of phytofluene and β-carotene, which were present in small amounts in the lycopene-containing treatments, increased significantly with ingestion of these products. Strong correlations were found between plasma and BMC concentrations of lutein, β-cryptoxanthin, α-carotene, and β-carotene. In contrast, correlations between lycopene concentrations in plasma and in BMCs were weak and not significant for any treatment.

Conclusions: The cellular content of lycopene and other tomato-related carotenoids with proposed beneficial health effects can be increased through prolonged supplementation. Am J Clin Nutr 1999;70:490–4.

KEY WORDS Lycopene, phytofluene, carotenoids, buccal cells, plasma, tomato juice, oleoresin, beadlets, humans

INTRODUCTION

Several epidemiologic studies have suggested a protective effect of lycopene and lycopene-rich foods, such as tomatoes, tomato sauce, and pizza, against certain cancers (1–5). To exert any effects in humans, lycopene must be absorbed by the intestine and distributed to the tissues via the circulation. Few studies have assessed the bioavailability of lycopene and, of those that have, results have been inconclusive, suggesting poor (6–8), moderate (9, 10), or good (11) bioavailability. Most of these studies measured plasma lycopene response as an indicator of bioavailability. However, plasma carotenoid concentrations do not necessarily reflect the amount of carotenoids absorbed or the concentrations in tissues. Therefore, it is important to monitor carotenoids in tissues concurrently with plasma to gain information on the distribution of carotenoids. One tissue, buccal mucosa cells (BMCs) from the inside of the cheeks, may be useful for monitoring tissue concentrations of carotenoids because the cells can be readily collected in a noninvasive manner.

In a previous report, we compared the plasma lycopene response produced by consumption of lycopene from a food source to that produced by lycopene supplements (12). Here, we extend those findings with results of a study on the effect of supplementation on the carotenoid concentrations in BMCs. We also examined correlations between plasma concentrations and BMC concentrations of various carotenoids.

SUBJECTS AND METHODS

Fifteen (9 female and 6 male) healthy subjects aged 33–61 years completed the study. Subjects were nonsmokers and were not taking vitamin-mineral supplements during the study. All procedures were approved by the Institutional Review Board of the Johns Hopkins Committee on Human Research. Subjects gave their written consent. The study design and protocol were described elsewhere (12). Briefly, lycopene was administered either in the form of lycopene-rich tomato juice [476 mL/d (≈2 cups) containing 74.9 mg lycopene], oleoresin soft-gel capsules (4 capsules/d

REFERENCE

1. From the US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Phytonutrients Laboratory, Beltsville, MD.

2. Reference to a company or product name does not imply approval or recommendation of the product by the US Department of Agriculture to the exclusion of others that may be suitable.

3. Supported by H Reisman Corp (Orange, NJ), which provided the lycopene beadlets. The other treatment preparations were supplied by LycoRed Natural Products Industries Ltd (Beer Sheva, Israel).

4. Address reprint request to BA Clevidence, USDA-ARS-BHNRC-PL, Building 308-East, Room 114A, Beltsville, MD 20705. E-mail: clevidence@bhnrc.arsusda.gov.

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myces griseus; Sigma Chemical Co, St Louis). After incubation for 4 wk, the cells were centrifuged, the supernate was discarded, the cells were packed in 1 mL physiological saline plus 100 μL 4 mol guanidinium isothiocyanate buffer/L. A 1:64 or 1:128 diluted cell suspension was used in the bicinchoninic acid protein assay (Micro BCA Protein assay; Pierce Chemical Co, Rockford, IL). Plasma carotenoids were extracted and quantified as described above and as described in greater detail previously (12).

Statistical analysis

Data were analyzed by using SIGMASTAT 2.03 (SPSS, Inc, Chicago) software. Descriptive statistics were used to compute means and SEMs. Significant differences from baseline and between treatments were measured by repeated-measures analysis of variance on log-transformed data. Tukey’s test was used to make pairwise comparisons when the F test result was significant. To assess the plasma-BMC relation of carotenoid concentrations, Spearman correlation coefficients were computed from data corresponding to a single day at the end of each treatment. A P value ≤0.05 was considered statistically significant for all tests.

RESULTS

The analytically determined quantities of carotenoids administered daily during the tomato juice, oleoresin, and beadlet treatments are presented in Table 1. The treatments were designed to provide substantially more lycopene than the estimated mean intake (2.6 mg/d) in the United States, but amounts provided were similar to estimated intakes of β-carotene (1.8 mg/d) and lutein (1.3 mg/d) (18). Lycopene in BMCs increased significantly from baseline, 2-fold after 4 wk of oleoresin and beadlet ingestion, to 4.95 and 3.75 μg/g protein, respectively, but was not significantly affected by tomato juice treatment (Table 2). The placebo treatment produced a significant decrease in BMC lycopene concentrations. Phytofluene, which was present in the lycopene-containing treatments, increased significantly with ingestion of tomato juice and oleoresin, but not with beadlet treatment. The beadlets provided less phytofluene than did the oleoresin or tomato juice (Table 1). A significant decrease in BMC phytofluene was observed after 4 wk of placebo treatment. The lycopene-containing treatments also provided 1.61–2.09 mg β-carotene. In response, the β-carotene content of BMCs increased significantly over baseline values with tomato juice, oleoresin, and beadlet treatments. Phytoene, although present in the treatments and plasma, was not detected in BMCs.

Lycopene increments (concentrations after 4 wk of supplementation corrected for baseline values) in BMCs were significantly different between the tomato juice and oleoresin treatments (P = 0.018), oleoresin and placebo (P < 0.001), and beadlets and

### Table 1

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Tomato juice</th>
<th>Oleoresin</th>
<th>Beadlets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carotenoid</strong></td>
<td><strong>mg/d</strong></td>
<td><strong>mg/d</strong></td>
<td><strong>mg/d</strong></td>
</tr>
<tr>
<td>Lycopene</td>
<td>74.90</td>
<td>75.40</td>
<td>70.20</td>
</tr>
<tr>
<td>Cyclolycopene</td>
<td>0.52</td>
<td>0.29</td>
<td>0.38</td>
</tr>
<tr>
<td>Lutein</td>
<td>1.87</td>
<td>1.66</td>
<td>1.20</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>2.09</td>
<td>2.01</td>
<td>1.61</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.54</td>
<td>ND²</td>
<td>0.37</td>
</tr>
<tr>
<td>Phytofluene</td>
<td>5.11</td>
<td>4.91</td>
<td>3.67</td>
</tr>
<tr>
<td>Phytoene</td>
<td>5.76</td>
<td>4.40</td>
<td>2.46</td>
</tr>
</tbody>
</table>

¹2,6-Cyclolycopene-1,5-diol.
²Not detected.
TABLE 2
Concentrations of carotenoids in buccal mucosa cells at baseline (week 0) and after 4 wk of treatment with 3 lycopene-containing products and placeboa

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Tomato juice</th>
<th>Oleoresin</th>
<th>Beadlets</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 4</td>
<td>P</td>
<td>Week 0</td>
</tr>
<tr>
<td>Lycopene (µg/g protein)</td>
<td>6.1 ± 0.38</td>
<td>3.26 ± 0.70</td>
<td>NS</td>
<td>2.05 ± 0.29</td>
</tr>
<tr>
<td>β-Carotene (µg/g protein)</td>
<td>0.90 ± 0.27</td>
<td>1.75 ± 0.39</td>
<td>0.003</td>
<td>1.04 ± 0.28</td>
</tr>
<tr>
<td>Phytofluene (µg/g protein)</td>
<td>0.97 ± 0.13</td>
<td>2.26 ± 0.26</td>
<td>&lt;0.001</td>
<td>0.84 ± 0.12</td>
</tr>
</tbody>
</table>

1 ± SEM; n = 15.
2 Change from week 0 in carotenoid concentrations.

placebo (P = 0.003) (Figure 1). There were no significant differences between the tomato juice treatment and the placebo. The increments of phytofluene in BMCs during tomato juice and oleoresin ingestion were significantly different from those during placebo treatment (P < 0.001 and P = 0.038, respectively; Figure 1).

Plasma concentrations of lycopene, β-carotene, and phytofluene at baseline and after 4 wk of supplementation are presented in Table 3. The mean plasma concentration-time curves for lycopene for all treatments are shown in Figure 2. At week 1, the plasma response during beadlet treatment was significantly higher than during the tomato juice treatment. Beyond week 2, the plasma lycopene response was not significantly different between the beadlet, oleoresin, or tomato juice treatments.

As shown in Table 4, plasma and BMC concentrations of lutein, β-cryptoxanthin, and β-carotene were significantly correlated during all treatment periods. The plasma-BMC relations for α-carotene were significant during oleoresin, beadlet, and placebo treatments but not during the tomato juice treatment. In contrast, the correlation between lycopene in BMCs and in plasma was not significant for any of the treatments, and correlations for lycopene were generally weaker than correlations for other hydrocarbon carotenones.

DISCUSSION

Bioavailability of food constituents is a complex issue involving digestion, intestinal uptake and absorption, distribution to the tissues, and utilization by the tissues. Studies addressing the bioavailability of carotenoids predominantly use plasma response as an indicator of bioavailability. The reasoning is that plasma concentrations of carotenoids reflect what is available to cells, where, presumably, carotenoids can be utilized and provide protection from disease. If, however, a compound is homeostatically regulated, the plasma concentration does not necessarily reflect how well the compound was absorbed, distributed to the tissues, and excreted. Concurrent assessment of changes in tissue and plasma concentrations of carotenoids in response to carotenoid intake is likely to be a more relevant estimate of bioavailability.

It was shown that concentrations of β-carotene in plasma and BMCs can be increased by β-carotene ingestion (19–22). Oral administration of β-carotene results in marked plasma responses (7), whereas lycopene ingestion produces no (6–8) or moderate responses (9–12). The moderate plasma response observed with supplementation of lycopene may be explained by rapid tissue uptake. Therefore, it is important to monitor changes in tissue lycopene concentrations, although limited availability of tissue biopsies makes this difficult. BMCs are a tissue that can be collected noninvasively and analyzed for carotenoid content (17, 22).

In this study, we showed that chronic ingestion of lycopene-containing products resulted in elevated lycopene concentrations in BMCs. The increase in BMC lycopene that occurred during tomato juice ingestion was not significant, whereas the changes during oleoresin and beadlet treatment were significant. Note, however, that lycopene concentrations in BMCs fell by about one-half after subjects consumed the placebo treatment. Thus, it appears that substantial amounts of lycopene were transported to BMCs after treatment not only with supplements but also with tomato juice. In contrast with BMCs, plasma concentrations of lycopene increased significantly after all 3 lycopene-containing treatments (Table 3). The most likely reason for this difference in plasma and BMC response is that the delayed increase in plasma concentrations of lycopene from tomato juice (Figure 2) limited the ability of this carotenoid to accumulate in BMCs. The plasma concentration-time curve for lycopene during tomato juice feeding was below those during the oleoresin and beadlet feedings throughout the study period; however, they were not significantly different beyond week 2. Because we collected BMCs only at baseline and after 4 wk of supplementation, we do not know the time required for BMC lycopene concentrations to begin to increase. It is possible that the increase caused by tomato juice would have been significant with a longer treatment period.
The BMC data suggest that elevated plasma lycopene concentrations must be present for a prolonged time before there is significant accumulation of lycopene in the superficial layer of the buccal epithelium, which we collected by scraping the insides of the cheeks. A factor that should be considered with regard to the observed effect is the turnover rate of BMCs. The buccal epithelium is a nonkeratinized tissue with a shorter turnover time than keratinized oral epithelium (23). Reported turnover times for buccal epithelium are not consistent and vary from 5 to 25 d (24, 25). Cells from the basal layer (stratum basale) migrate through the prickle cell and intermediate layer to the superficial layer (stratum distendum). During the tomato juice treatment period, the cells of the basal layer were exposed to elevated plasma lycopene concentrations, comparable with those achieved during beadlet and oleoresin treatments, for 14 d (weeks 2–4). During the beadlet and oleoresin treatments, plasma concentrations were elevated for 21 d. A turnover rate for BMCs > 14 d, ie, the time it takes basal cells to become superficial cells, would imply that the buccal cells of the basal layer must be subjected to high plasma lycopene concentrations before week 2 to see a significant rise in the cellular lycopene content of the superficial layer at week 4.

We observed a moderate but significant increase from baseline of plasma lycopene concentrations with tomato juice, oleoresin, and beadlets treatments: 42%, 40%, and 41% at 4 wk, respectively. In fact, plasma lycopene concentrations plateaued at ≈2 wk of supplementation; from that point on, there were no significant differences between treatments. The moderate plasma lycopene response, relative to that of other common carotenoids, may have been due to one or a combination of several factors: 1) decreased absorption of lycopene by the intestine, 2) increased excretion of lycopene through the bile, or 3) increased tissue uptake of lycopene. We found a 2.5- and 2-fold increase of BMC lycopene during oleoresin and beadlet treatment, respectively. Tomato juice ingestion produced a 25% increase in BMC lycopene concentrations; however, this change was not significant. This finding was unexpected. Not only were plasma concentrations of lycopene not significantly different by treatment, but the distribution of lycopene among plasma lipoproteins, the carriers of carotenoids to tissues, also did not differ significantly (12). A 2-fold increase in cellular lycopene content does not seem extraordinary and increased tissue uptake is unlikely to be responsible for the small increments in plasma lycopene observed in this and other studies. Other carotenoids, such as β-carotene, have been shown to increase substantially in plasma and BMCs concurrently with ingestion (19, 22). Of course, tissues other than BMCs may selectively accumulate more lycopene.

Because BMCs can be collected by noninvasive procedures, the strength of the correlation between plasma and BMC carotenoid concentrations is of interest. If carotenoid concentrations in BMCs accurately reflect plasma concentrations, it will no longer be necessary to draw a blood sample to evaluate the carotenoid status of individuals. Results from previous studies are contradictory, suggesting no correlation (26) or a good correlation (17) between plasma and BMC carotenoids. Here, we showed a significant correlation between plasma and BMC concentrations of lutein, β-cryptoxanthin, α-carotene, and β-carotene, suggesting that cellular carotenoid concentrations are good biomarkers for plasma concentrations of these carotenoids. Interestingly, such correlations for lycopene were weak and not significant for any of the treatments. Thus, unlike most of the major dietary carotenoids, lycopene in BMCs does not reflect plasma lycopene concentrations.

This study showed that carotenoid concentrations in BMCs can be significantly increased by prolonged (4 wk) intake of tomato-derived products. Tissue uptake and utilization of carotenoids are important aspects to consider when addressing their bioavailability.

![FIGURE 2. Mean (±SEM) increments in plasma lycopene concentration-time curves (n = 15) at baseline and during 4 wk of intervention with lycopene beadlets (•), oleoresin (□), tomato juice (◇), or placebo (▲). Concentrations were adjusted by subtracting baseline values. Different letters indicate significant differences between treatments at each time point.](image-url)
An important finding of this study was the strong correlations between tissue and plasma concentrations for several major carotenoids. The notable exception was lycopene, the focus of this study, for which there was not a significant correlation between plasma and BMC concentrations. It appears that buccal cell carotenoid concentrations reflect plasma concentrations of most but not all of the major dietary carotenoids.

We thank Evelyn Lashley and her staff for food procurement, preparation, and service; the study participants for their efforts; Benjamin Caballero and staff, Johns Hopkins School of Public Health, Baltimore, for medical supervision of the study; and James C Smiths Jr and Joseph T Judd for their helpful advice in the preparation of the study.

REFERENCES


*Table 4* Spearman rank order correlations of carotenoid concentrations in plasma and buccal mucosal cells after the 3 lycopene treatments and placebo.

<table>
<thead>
<tr>
<th></th>
<th>Tomato juice</th>
<th>Oleoresin</th>
<th>Beadlets</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>0.650</td>
<td>0.873</td>
<td>0.790</td>
<td>0.781</td>
</tr>
<tr>
<td>α-Cryptoxanthin</td>
<td>0.110</td>
<td>0.345</td>
<td>0.337</td>
<td>0.242</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.815</td>
<td>0.597</td>
<td>0.707</td>
<td>0.728</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.204</td>
<td>0.332</td>
<td>0.032</td>
<td>0.414</td>
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<tr>
<td>α-Carotene</td>
<td>0.474</td>
<td>0.796</td>
<td>0.581</td>
<td>0.728</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.613</td>
<td>0.720</td>
<td>0.617</td>
<td>0.775</td>
</tr>
<tr>
<td>Phytolfluene</td>
<td>0.336</td>
<td>0.627</td>
<td>0.318</td>
<td>0.581</td>
</tr>
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</table>

1. n = 15.
2. P < 0.01.
3. P < 0.001.
4. P < 0.05.