ABSTRACT This study investigated the postprandial plasma responses of carotenoids for 24 h after feeding five breakfast beverages; four of which had low or no carotenoid content. In seven fasting healthy elderly female subjects a blood sample (baseline) was obtained, after which they were given a breakfast beverage, containing one of the following: 1) strawberries (240 g); 2) ascorbic acid (1250 mg); 3) spinach (294 g); 4) red wine (300 mL); and 5) control (breakfast beverage only). Blood samples were collected at 0.5, 1, 4, 7, 11, 15, and 24 h. Plasma carotenoids were measured using HPLC. No significant differences were found in the levels of the plasma carotenoids measured among the various treatments at baseline. In the spinach treatment, plasma lutein, zeaxanthin and β-carotene levels at 7, 11, 15 and 24 h were significantly higher than those at baseline, as expected. All of the carotenoids measured in the control and vitamin C treatments, at subsequent sampling times were not significantly different from those at baseline. However, for most carotenoids, strawberry and red wine feeding resulted in significantly lower carotenoid values from baseline at 11 and 15 h. Subjects who received a diet with low levels of carotenoids, but whose postprandial plasma levels of carotenoids remain steady, might be explained by a mechanism that promotes secretion of carotenoids into the circulation. Assuming that plasma carotenoids are being used over time, we hypothesize that strawberries and red wine contain some substances that interfere with the secretion of carotenoids into the circulation. J. Nutr. 128: 2391–2394, 1998.

KEY WORDS: carotenoids • postprandial • vegetables • fruits • red wine • humans

Consumption of fruits and vegetables has been associated with protection against various diseases, including cancer and cardiovascular and cerebral-vascular diseases (Colditz et al. 1985, LaVecchia et al. 1998, Longnecker et al. 1997, Steinmetz and Potter 1996). Plasma carotenoid concentrations are negatively correlated with the risk for cardiovascular disease and cancer, and carotenoids may therefore in part mediate the protective effect of vegetables and fruits.

In subjects fed a low carotenoid diet for 13 wk, a decline in the plasma carotenoid levels was observed, suggesting that carotenoid levels reflect recent dietary intake (Rock et al. 1992). Dietary intakes of carotenoids, calculated from vegetable and fruit intakes expressed in servings per day, generally reflect plasma concentrations (Polsinelli et al. 1998). The recommendation of the 1990 Dietary Guidelines for Americans and the Food Guide Pyramid is that people consume five or more servings of fruits and vegetables each day. However, fewer than one-third of American adults follow this recommendation (Anonymous 1995).

There are few data in the literature on the metabolism of stored carotenoids in a postprandial situation after consuming diets of low carotenoid content. Brown et al. (1989) studied the response of individual carotenoid concentrations for a 4-h period after ingestion of a low carotenoid meal and did not observe any significant change in their values. The postprandial period is important to study because most of our lives are spent in the postprandial state, and postprandial dyslipidemia is a known risk factor for coronary heart disease (Gylling and Miettinen 1993).

We conducted this study to obtain information about carotenoids in plasma in the postprandial state of subjects receiving several different low carotenoid diets or spinach. Our study was part of a bigger study that was designed to compare changes in plasma oxygen radical absorbance capacities (ORAC)6 (Cao et al. 1995), following a meal containing strawberries, spinach, red wine or vitamin C (Cao et al. 1998).

SUBJECTS AND METHODS

Subjects. Eight healthy female subjects age 66.9 ± 0.6 y were recruited to participate in this study (one subject did not finish all experiments). The characteristics of the subjects have been reported elsewhere (Cao et al. 1998). The study protocol was approved by the

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5 To whom correspondence should be addressed.
6 Abbreviations used: AUC, area-under-curve; HDL-C, HDL-cholesterol; ORAC, oxygen radical absorbance capacity.

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Human Investigation Review Committee of Tufts University and the New England Medical Center, and written informed consent was obtained from each study participant.

Study design. Each subject was asked to come to the Metabolic Research Unit at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University in the evening and to fast overnight. In the morning, an intravenous needle was inserted into one forearm of each subject. A 10 mL blood sample (zero baseline sample) was obtained from fasting subjects, following which they consumed a glass of breakfast beverage, 378 mL of coconut drink (Cao et al. 1998), containing one of the treatments assigned in a random sequence to each subject. Treatments were provided daily allowance for protein and energy (Cao et al. 1998). Additional blood samples were obtained at 7, 11, 15 and 24 h following the blood sampling at 4 h, a low carotenoid lunch meal was given (Cao et al. 1998). Additional blood samples were obtained at 7, 11, 15 and 24 h following the initial blood sampling. A snack was given after the 7 h blood sampling and a low carotenoid dinner was given immediately after the 11 h blood sampling (Cao et al. 1998). The breakfast, lunch and dinner were designed to be low in foods containing antioxidant activity [i.e., no significant amounts of carotenoids (<15 μg), α-tocopherol, flavonoids and vitamin C], but to provide the recommended daily allowance for protein and energy (Cao et al. 1998). Each subject received each of the five treatments (drinks) on a separate day, 2 wk apart, during a period of 8 wk. Treatments were assigned in a random sequence to each subject.

Controlled diets. All nutrient determinations were obtained from calculations done using the Minnesota Nutrient Data System software (Food data base, version 9A; Nutrient Database, version 2.7; Nutrition Coordination Center, University of Minnesota, MN). The carotenoid concentrations of the diets were determined by using individual aliquots of the replicate strawberry and spinach homogenates and red wine using the modified official method of analysis of the Association of Official Analytical Chemists (Deutsch 1984) and analyzed with the high performance liquid chromatography (HPLC) system used for plasma analysis.

Plasma analysis. Lutein, zeaxanthin, cryptoxanthin, α-carotene, β-carotene and lycopene were analyzed by an HPLC system that consisted of a series 410 LC pump (Perkin-Elmer, Norwalk, CT), a Waters 717 plus autosampler (Millipore, Milford, MA), a C30 carotenoid column (3 μm, 150 x 4.6 mm, YMC, Wilmington, NC), an HPLC column temperature controller (model 7950; column heater/chiller, Jons Chromatography, Lakewood, CO), a Waters 994 programmable photodiode array detector and a Waters 840 digital 350 data station according to the method of Yeum et al. (1996). The total carotenoid level represents the sum of each of the individual carotenoid levels (lutein, zeaxanthin, cryptoxanthin, α-carotene, cis- and trans-β-carotene, cis- and trans-lycopene) as measured in the sample.

Total cholesterol, triglycerides and high density lipoprotein-cholesterol (HDL-C) were determined in plasma at all eight time points of the control, strawberry and red wine treatments. In vitamin C and spinach treatments these substances were not measured because of insufficient samples. Cholesterol and triglycerides concentrations were determined with a colorimetric method (Bucolo and David 1973) by using Roche Reagents (Roche Diagnostic Systems, Nutley, NJ). HDL-C concentrations were determined by a colorimetric method using HDL Direct Cholesterol Reagent (Equal Diagnostics, Exton, PA). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol were calculated using the formula of Friedewald et al. (1972).

Statistical analysis. To standardize the presentation, results are expressed as percentage of concentrations at baseline. Comparisons among the five treatments at baseline (time 0) were made by repeated measures ANOVA. Multiple comparison procedures were made using the Tukey test. When data showed a nonnormal distribution, comparisons were made using Friedman repeated measures analysis. Multiple comparison procedures were made using Student-Newman-Keuls’s Method. Repeated measures ANOVA or Friedman’s nonparametric ANOVA were used to compare the responses to the treatments. If a difference was found within the treatment, we performed multiple comparisons between time pairs, using the Student-Newman-Keuls test. Statistical significance was set at the P < 0.05 level. Values are presented as means ± SEM. To represent the statistical values in the graphics, a scatter plot with SEM bars was used. Data analysis was carried out with SigmaStat version 2.0 for Windows 95, NT & 3.1 (Jandel Scientific Software, San Rafael, CA).

RESULTS

No significant differences were found in the baseline plasma levels of lutein, zeaxanthin, cryptoxanthin, α-carotene, β-carotene, lycopene and total carotenoids among the treatments (data not shown).

All of the carotenoids measured at all sampling times after the control treatment (0.5, 1, 4, 7, 11, 15 and 24 h) were not significantly different from the baseline (Fig. 1). The responses in plasma lutein, zeaxanthin, cryptoxanthin, α-carotene, β-carotene, lycopene and total carotenoids following the consumption of the breakfast drink containing spinach are shown in Figure 2. All subjects had an increase in the values of lutein, zeaxanthin and β-carotene after the beverage. Plasma lutein, zeaxanthin, β-carotene and total carotenoids levels at 7, 11, 15 and 24 h were significantly higher than those at baseline. Plasma lycopene values at 7, 11, 15 and 24 h were significantly lower than those at baseline (P < 0.05). Plasma cryptoxanthin and α-carotene values at 0.5, 1, 4, 7, 11, 15 and 24 h were not significantly different than those at baseline. The mean values of area under the curve (AUC) for the plasma lutein response, were 1.4 times higher than the AUC for zeaxanthin and β-carotene response. However, when these values were corrected by the amount of each carotenoid ingested in the breakfast beverage (Table 1), zeaxanthin showed the highest response (~30 times higher than for lutein and β-carotene).

The responses in plasma lutein, zeaxanthin, cryptoxanthin, α-carotene, β-carotene, lycopene and total carotenoids following the consumption of the strawberry breakfast drink are shown in Figure 1. For most all carotenoids measured, strawberry consumption resulted in significantly lower values than baseline at sampling times 11 and 15 h. However, β-carotene

### Table 1

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Spinach</th>
<th>Strawberry</th>
<th>Red wine</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount1, g</td>
<td>294</td>
<td>240</td>
<td>3003</td>
<td>1,250</td>
</tr>
<tr>
<td>Energy1, kJ</td>
<td>270.7</td>
<td>301.2</td>
<td>300</td>
<td>16.8</td>
</tr>
<tr>
<td>Protein1, g</td>
<td>8.41</td>
<td>1.46</td>
<td>8.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Fat1, g</td>
<td>1.03</td>
<td>0.89</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>β-carotene1, mg</td>
<td>14.79</td>
<td>0.038</td>
<td>14.14</td>
<td>1.95</td>
</tr>
<tr>
<td>Lutein2, mg</td>
<td>17.03</td>
<td>0.04</td>
<td>14.14</td>
<td>0.39</td>
</tr>
<tr>
<td>Zeaxanthin2, mg</td>
<td>0.39</td>
<td>nd4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>cis-β-carotene2, mg</td>
<td>1.95</td>
<td>nd</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lycopene2, mg</td>
<td>nd</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Nutrient determinations were obtained from calculations, using Minnesota Nutrient Data System software.
2 Nutrient determined by HPLC analysis.
3 The amount of red wine given was in mL.
4 nd = not detected.
was significantly different only at 15 h, and \( \alpha \)-carotene was not significantly different from the baseline values at any of the sampling times. The greatest reduction from baseline in percentages were 16.1, 14.1, 13.2 and 9.4% at 15 h for zeaxanthin, lycopene, lutein, \( \beta \)-carotene, respectively; and 12.1 and 10.8% at 11 h for cryptoxanthin and total carotenoids, respectively.

The responses in plasma lutein, zeaxanthin, cryptoxanthin, \( \alpha \)-carotene, \( \beta \)-carotene, lycopene and total carotenoids following the consumption of the red wine breakfast beverage are shown in Figure 1. Plasma cryptoxanthin, lycopene, total carotenoids and \( \beta \)-carotene values from the sampling times at least at 7, 11 and 15 h were significantly different than those at baseline (\( P < 0.05 \)). The greatest reduction from baseline in percentages were 13.9 and 12.5% for cryptoxanthin and lycopene at 15 h, respectively, and 11.0 and 9.9% for total carotenoids and \( \beta \)-carotene at 7 h, respectively. Plasma lutein, zeaxanthin and \( \alpha \)-carotene measured at the various sampling times were not significantly different than those at baseline. All of the carotenoids measured at the sampling times after the vitamin C treatment were not significantly different than those at baseline, although values were reduced at 15 h. The reduction from baseline in percentages at time 15 h were 9.4, 9.1, 8.6, 8.5, 7.2, 6.4 and 6.2% for lycopene, zeaxanthin, cryptoxanthin, lutein, \( \alpha \)-carotene, total carotenoids and \( \beta \)-carotene, respectively.

The plasma LDL cholesterol responses following the consumption of the control, strawberry and red wine breakfast drinks are shown in Figure 2. Values were significantly different from baseline beginning at 1 h after the consumption of the strawberry beverage and at 11 and 15 h after consumption of the control beverage. The reduction from the baseline in percentages were 14.1 and 13.0% for strawberry and control treatments, respectively.

**DISCUSSION**

Carotenoids are absorbed by small intestinal mucosal cells by a mechanism involving passive diffusion similar to that for cholesterol and products of triglyceride lipolysis (Parker 1996). In humans, carotenoids are transported in blood exclusively via lipoproteins. Under fasting conditions, up to 75% of plasma hydrocarbon carotenoids are found in LDL fractions and most of

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**FIGURE 1** Changes from baseline in individual carotenoids in plasma of elderly women after ingestion of the control, strawberry or red wine breakfast beverages during a period of 24 h (Means ± SEM, \( n = 7 \)). The dotted line represents the no change line. The meal times are presented as dotted-dash lines. The \( P \) value for the repeated measure ANOVA or Friedman test in the control treatment were NS to all carotenoids measured, strawberry treatment were <0.02 at most to all carotenoids measured, red wine treatment were NS for lutein and zeaxanthin, and were <0.006 at most to \( \beta \)-carotene, lycopene and total carotene. The black symbols represent a significant difference from the baseline (\( P < 0.05 \)).

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**FIGURE 2** Changes from baseline in individual carotenoids in plasma of elderly women after ingestion of the spinach breakfast beverage during a period of 24 h and changes in LDL cholesterol from baseline in plasma after ingestion of the control, strawberry or red wine breakfast beverages during a period of 24 h (Mean ± SEM, \( n = 7 \)). The dotted line represents the no change line. The meal times are presented as dotted-dash lines. The \( P \) value for the repeated measure ANOVA or Friedman test in the spinach treatment were <0.001 for all carotenoids measured, and changes in LDL-cholesterol were <0.005 for control, strawberry and red wine treatments. The black symbols represent a significant difference from the baseline (\( P < 0.05 \)).
the remaining carotenoids are associated with HDL and, to a lesser degree, with VLDL (Erdman et al. 1993). Carotenoids appear initially in blood chylomicron and VLDL fractions followed sequentially by appearance in the LDL and the HDL fractions (Johnson and Russell 1992). Carotenoid distribution among various lipoprotein particles in the postprandial state are the result of numerous factors: absorption (Erdman et al. 1993), intraluminal interaction between the dietary carotenoids (Johnson et al. 1997, Kostic et al. 1995, Paetau et al. 1997), conversion of certain carotenoids to vitamin A (Solomons and Bulux 1993), packaging and transport by the lipoproteins (Parker 1996), and metabolism and release by the extra hepatic tissues. Also, plasma levels of certain carotenoids are influenced by different physiologic and lifestyle factors including sex, age, body mass index and smoking (Vogel et al. 1997).

Subjects fed the low carotenoid control diet had flat curves, i.e. no differences between the subsequent values and the baselines values (Fig. 1). Supplementing the breakfast beverage with spinach, a food that is rich in lutein, zeaxanthin and b-carotene, as expected, resulted in an increase in the plasma values of those carotenoids from 7 h post dose onward. This response occurred in all subjects. Our observation period was not long enough to observe a peak for any carotenoid after feeding the spinach meal. Peak blood responses of carotenoids after meal vary greatly. For example, after a single dose of b-carotene, the plasma concentration apogee point varied from 8–48 h (Solomons and Bulux 1993). Lutein showed the greatest change (rise) from baseline after spinach consumption, although when corrected for the amount of the carotenoids ingested in the spinach meal, the lutein and b-carotene responses were similar.

When supplementing the control diet with strawberries, red wine or vitamin C, with low levels or no carotenoids (Table 1), it was expected that the subjects would behave in the same way as when they received the unsupplemented control diet, i.e. they would show the same flat response curve for all measured carotenoids. However, we observed quite a different response (Fig. 1). The reason for these postprandial declines in the plasma carotenoids after the strawberry, red wine and vitamin C diets is unknown.

As shown in Table 1, the macronutrient content (energy, protein and the amount of fat) of the diets can not explain the different carotenoid response curves because the red wine and vitamin C supplements did not add a significant amount of energy, fat or protein to the control diet. Another possible explanation could be an interference of strawberries, red wine and vitamin C in lipoprotein metabolism. As already mentioned, in the fasting state LDL particles are the major carriers of plasma b-carotene (Erdman et al. 1993). In Figure 2, we have shown a significant decrease of LDL in the two treatments (control, and strawberry) at 15 h (4 h after the dinner meal). It is known that postprandial lipid, lipoprotein and apolipoprotein concentrations are affected by circadian factors, e.g. lower LDL and HDL response curves after a night time meal versus after a day time meal (Romon et al. 1997). The LDL observations in our study suggest that the differences between the control treatment and the strawberry and red wine treatments is not due to alterations in LDL concentrations. Thus subjects who receive a diet with low levels of carotenoids, but whose postprandial plasma levels of carotenoids remain steady, must have a mechanism that promotes secretion of carotenoids into the circulation. It has been reported that individuals receiving a diet practically devoid of carotenoids maintain steady plasma b-carotene concentrations for a two-week period (Johnson and Russell 1992). This observation supports the notion of a regulation of plasma carotenoid levels that would involve secreting carotenoids from tissues into the circulation because plasma carotenoids are being continuously metabolized. From this work, we can hypothesize that strawberries and red wine contain some substances that interfere with the secretion of carotenoids into the circulation.

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LITERATURE CITED


