

Effect of alcohol consumption on plasma carotenoid concentrations in premenopausal women: a controlled dietary study^{1,2}

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ABSTRACT This 6-mo controlled dietary study compared the effect of 30 g alcohol/d for three menstrual cycles with three alcohol-free cycles on plasma carotenoid concentrations in 18 nonsmoking, premenopausal women. Participants were randomly allocated within a crossover design to either phase and consumed ≈ 6 mg total carotenoids/d under isoenergetic conditions. Blood was drawn during the third menstrual cycle of each alcohol phase. After adjustment for the mean daily specific carotenoid and energy intakes for each alcohol phase, the paired differences in mean plasma α - and β -carotene concentrations were significantly higher by 19% ($P = 0.027$) and 13% ($P = 0.034$), respectively, during the alcohol-intake phase of the study. The paired difference in mean plasma lutein/zeaxanthin concentration was significantly lower by 17% ($P = 0.031$) when the participants consumed alcohol than when they did not. This is the first reported study in women to document the independent effect of alcohol on plasma carotenoid concentrations without the potential interaction of smoking under controlled dietary conditions. *Am J Clin Nutr* 1995;62:131-5.

KEY WORDS Carotenoids, alcohol, controlled diet, premenopausal women

INTRODUCTION

Plasma β -carotene concentrations are inversely associated with risk of lung and stomach cancers (1-4). The relation of alcohol to lung cancer is less consistent (5, 6). Because plasma β -carotene concentrations vary by alcohol intake, the relation between plasma carotenoid concentrations and cancer may be confounded by or modified by alcohol intake (7-9).

An inverse relation between alcohol consumption and plasma β -carotene concentrations in the free-living state is stronger in men than in women (8, 9). Although these correlations are adjusted for smoking status, they are not adjusted for dietary carotenoid intake, which could confound or modify this association. Therefore, the role of alcohol in the plasma carotenoid-cancer relation and its effect on individual plasma carotenoid concentrations is unclear.

Alcohol intake raises plasma high-density-lipoprotein-cholesterol (HDL) concentrations and lowers plasma low-density-lipoprotein-cholesterol (LDL) concentrations (10). The major carotenoids in the circulating plasma are transported on the HDL and LDL fractions. Approximately 70% of lycopene and β -carotene is transported on the LDL fraction and 30% is transported on the HDL fraction, whereas lutein is transported equally on the HDL and the LDL fractions (11). Therefore, alcohol intake may raise plasma carotenoids carried on the HDL fraction and lower those carried on the LDL fraction.

Alcohol can be a source of oxidative stress. Specific carotenoids, in their role as antioxidants, may be reduced in the presence of alcohol whereas oxidative byproducts (eg, ketocarotenoids) may appear at higher concentrations in the circulating plasma (12).

A controlled dietary study of alcohol intake and hormone concentrations was conducted in 1989 among 37 nonsmoking, premenopausal women (13). The data from this study were used to answer the following question: What is the effect of the daily intake of 30 g alcohol on plasma concentrations of individual and total carotenoids, tocopherols, and retinol in contrast with plasma concentrations during an alcohol-free phase?

SUBJECTS AND METHODS

Subjects

The study volunteers were female nonsmokers aged 21-39 y who passed the screening physical exam, with normal biochemistry indexes (13). They also met the following eligibility criteria: 1) they were healthy, with no history of chronic disease; 2) they were not pregnant, were not taking oral contraceptives or other hormone preparations, and had not been breast-feeding for the 12 mo preceding the study; 3) they had no reported history of menstrual irregularities and a reported menstrual cycle length of < 35 d; 4) they had not been taking medications except for analgesics; 5) they had no reported history of binge drinking or restricted dietary practices; and 6) they weighed between 80% and 130% of healthy weight based

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on the 1983 Metropolitan Life Insurance Table (13). Informed consent was obtained from all subjects, and all procedures were approved by the institutional review boards of the Georgetown University Medical School and the National Cancer Institute.

Of the 37 participants in the controlled diet study, 18 had confirmed ovulatory cycles in and had available plasma samples from the third and sixth menstrual cycles. Plasma samples were analyzed to determine carotenoid, tocopherol, and retinol concentrations. These 18 subjects had baseline characteristics similar to those of the original sample of 37 (Table 1).

Controlled diet study

Subjects were enrolled in the controlled dietary study on the same calendar day, not menses day, for six complete menstrual cycles ($n = 15$ who had ≤ 20 d before day 1 of the first cycle of menses). The participants were randomly allocated to consume 30 g alcohol/d for three menstrual cycles, followed by three alcohol-free cycles, or the reverse. The controlled diet study was conducted at the US Department of Agriculture (USDA), Beltsville Human Nutrition Research Center (BHNRC), Beltsville, MD, and consisted of a 14-d repeat menu cycle typical of the American diet. The daily diet of energy was distributed as 1) 36% of energy from fat, 2) 53% of energy from carbohydrates, 3) 14% of energy from protein, 4) 0.348 g fiber/kJ, and 4) 0.630 mg cholesterol/kJ. All meals were prepared and eaten at the BHNRC, except for the carry-out lunches on weekdays and the carry-out meals on weekends and holidays.

To maintain isoenergetic conditions, the participants were weighed daily before breakfast. If a woman lost or gained ≥ 1 kg and maintained that weight for ≥ 3 d, her energy intake was increased or decreased in increments of 47.6 kJ (200 kcal). Vitamin and mineral supplements were prohibited throughout the controlled dietary study. Alcohol was consumed in the form of 95% alcohol in fruit juice before bedtime; subjects were restricted from driving a vehicle after consumption (13). No other alcohol consumption was permitted during the study other than that provided. During the alcohol-free phase of the study, energy-equivalent amounts of soft drinks were substituted for the alcohol and fruit juice.

Measurement of dietary carotenoid intake

All carotenoid-rich foods from each daily menu were linked to values for five carotenoids (α -carotene, β -carotene, lutein/zeaxanthin, β -cryptoxanthin, and lycopene) on the USDA-National Cancer Institute Carotenoid Food Composition

TABLE 1

Characteristics of the current sample compared with the total number of participants in the controlled dietary study¹

Characteristic	Current sample ($n = 18$)	Total ($n = 37$)
Age (y)	30 \pm 5	30 \pm 5
BMI (kg/m ²)	25 \pm 4	24 \pm 5
Plasma cholesterol (mmol/L)	4.76 \pm 0.88	4.71 \pm 0.83
Reported drinks/wk ²	1.6 \pm 2.4	1.7 \pm 1.4

¹ $\bar{x} \pm$ SD.

² The average alcohol intake was based on 7 consecutive days of food diaries during the free-living phase of the study.

Database (14). Specific and total carotenoid intakes were estimated daily for each subject during each phase.

Measurement of plasma carotenoid, hormone, cholesterol, and lipid concentrations

Approximate 12-h fasting blood samples were collected and immediately centrifuged at $1500 \times g$ for 20 min at 4 °C, and plasma was stored in plastic tubes containing EDTA in the early and late follicular and luteal phases (days 5–7, 12–15, and 21–23) in the third and sixth menstrual cycles. 24-h urine specimens were collected over the same days.

Because serum β -carotene and several lipid components are reported to fluctuate by phase of the menstrual cycle (15, 16), only women with confirmed ovulatory cycles were selected and plasma specimens were selected for the same days of the cycle from each woman. Ovulation was determined from urinary luteinizing hormone (LH) concentrations in the late follicular phase and serum progesterone concentrations in the luteal phase (13). Plasma samples from day 5 of menstrual cycles three and six were analyzed enzymatically for cholesterol and triglycerides with ABA-100 analyzers (Abbott, Abbott Park, IL) by using reagents supplied by Abbott Diagnostics, Chicago. HDLC was analyzed similarly by using heparin-manganese (17), whereas the Friedewald formula (18) was used to estimate LDLC concentrations.

Plasma specimens from 1 to 3 consecutive days during days 12–15 of menstrual cycles three and six were available for the analysis of plasma carotenoid, tocopherol, and retinol concentrations. These specimens were stored at -70 °C for ≤ 2 y. Individual daily plasma α -carotene, β -carotene, cryptoxanthin, lutein/zeaxanthin, anhydrolutein, lycopene, α - and γ -tocopherol, and retinol concentrations were analyzed by HPLC using a C₁₈ reversed-phase column as described by Khachik et al (19). Pure forms of each carotenoid were used as reference standards for quantitation. All samples of each subject were analyzed in one batch on the same day to minimize day-to-day variation, and plasma concentrations of each subject in each alcohol phase were averaged.

Statistical analysis

In the initial analysis, measures of central tendency were calculated for each alcohol phase of the study for plasma concentrations of the specific and total carotenoids, tocopherols, retinol, cholesterol, and lipid components, and for dietary intakes of specific and total carotenoids and total energy.

After the initial analysis, the unadjusted and adjusted paired differences (within-person) in mean plasma carotenoid, tocopherol, and retinol concentrations were computed by using analysis of variance (ANOVA) with SAS, PROC GLM (SAS Institute, Cary, NC). Because the women were enrolled by calendar, not menses, day in the study, the average daily specific and total carotenoid intakes and total energy intakes varied by alcohol phase of the study. Therefore these two dietary factors and two other variables, a person-level fixed effect and alcohol phase, were included for adjustment in the ANOVA models. Average daily intakes of carotenoids and energy were treated continuously and the person effect and alcohol phase were treated categorically. The person-level fixed effect created an intercept for each person, which was equivalent to using the ANOVA as a matched paired *t* test.

The advantage of the ANOVA statistic was the opportunity to use different multiples of consecutive days of plasma per person in each alcohol phase (ie, 12 with two specimens and 1 with three specimens for each alcohol phase; 4 with two specimens for one alcohol phase and one specimen for the other phase; and 1 with one specimen for each phase). The ANOVA weighted the mean plasma concentrations to take into account the effect of unbalanced sets of daily measurements on the precision around the mean. Results did not differ when the data for the five subjects with one specimen in each alcohol phase were removed; therefore, the analysis of the 18 participants was presented. All findings were considered statistically significant at a P value ≤ 0.05 .

RESULTS

The mean individual and total dietary carotenoid intakes did not significantly differ by phase of alcohol intake (Table 2). Mean plasma HDLC was significantly higher (by 8%) and mean plasma LDLC was significantly lower (by 11%) during the alcohol-intake phase than during the alcohol-free phase.

Mean plasma α - and β -carotene, lycopene, total carotenoids, and retinol concentrations were higher when the subjects were consuming alcohol than when not; however, only the paired difference in the unadjusted mean plasma α -carotene concentration was statistically significant ($F = 18.12$) (Table 3).

After adjustment for total energy intake and intake of the specific carotenoid by phase, the paired difference in mean plasma α - and β -carotene concentrations was significantly higher (by 19% and 13%, respectively) when the subjects were consuming alcohol than when not (Table 4). In contrast, the adjusted paired difference in the mean plasma lutein/zeaxanthin concentrations was significantly lower (by 17%) during the alcohol phase than during the alcohol-free phase. No other differences were statistically significant.

DISCUSSION

Under controlled dietary conditions, mean plasma α - and β -carotene concentrations were significantly higher whereas mean plasma lutein/zeaxanthin concentrations were significantly lower with alcohol consumption during the three menstrual cycles than

TABLE 2

Dietary intake of individual and total carotenoids, and plasma HDL and LDL cholesterol concentrations by alcohol phase of the controlled dietary study¹

Dietary carotenoids	Alcohol phase	Alcohol-free phase
	<i>mg/d</i>	
α -Carotene	0.4 \pm 0.6	0.5 \pm 0.6
β -Carotene	1.9 \pm 0.2	2.0 \pm 0.3
β -Cryptoxanthin	0.1 \pm 0.01	0.1 \pm 0.01
Lutein/zeaxanthin	2.1 \pm 0.3	2.2 \pm 0.3
Lycopene	1.2 \pm 0.1	1.2 \pm 0.2
Total carotenoids	5.7 \pm 0.7	6.0 \pm 0.9
Plasma HDL cholesterol	1.58 \pm 0.34	1.47 \pm 0.39 ²
Plasma LDL cholesterol	2.33 \pm 0.75	2.51 \pm 0.67 ²

¹ $\bar{x} \pm$ SD; $n = 18$.

² Significantly different from alcohol phase, $P < 0.001$.

TABLE 3

Specific and total plasma carotenoid, tocopherol, and retinol concentrations, by phase of the controlled dietary study¹

Plasma carotenoids, tocopherol, and retinol	Alcohol phase	Alcohol-free phase
	<i>μmol/L</i>	
α -Carotene	17.4 \pm 12.6	14.2 \pm 9.6 ²
β -Carotene	762.6 \pm 521.6	707.9 \pm 428.5
Cryptoxanthin	651.6 \pm 362.0	760.2 \pm 398.2
Lutein/zeaxanthin	774.4 \pm 264.0	862.4 \pm 334.4
Anhydrolutein	193.6 \pm 70.4	193.6 \pm 70.4
Lycopene	1302.0 \pm 576.6	1246.2 \pm 558.0
Total carotenoids	3293.0 \pm 1332.0	3237.5 \pm 1239.5
α -Tocopherol	23.2 \pm 7.0	23.2 \pm 7.0
γ -Tocopherol	4.6 \pm 0.2	4.6 \pm 0.2
Retinol	2.4 \pm 0.8	2.3 \pm 0.6

¹ $\bar{x} \pm$ SD; $n = 18$.

² Significantly different from alcohol phase, $P = 0.02$.

during three alcohol-free cycles. All findings were adjusted for total energy intake and dietary intake of the specific carotenoid by phase of alcohol intake. There was no evidence of a crossover effect.

In a controlled diet study of baboons fed either 200 g carrots/d or a pharmaceutical dose of 30–45 mg β -carotene/d, mean plasma β -carotene concentrations were higher and its clearance from the plasma was delayed in baboons who were fed alcohol as isoenergetic replacement for carbohydrates compared with age- and body weight-matched control subjects (20). A similar positive relation between alcohol intake and plasma α - and β -carotene concentrations in men appeared at hospital admission for alcohol rehabilitation (21). In contrast with these heavy drinking conditions, daily alcohol intake was considerably lower in the current study, nonetheless, the effect of alcohol on plasma α - and β -carotene concentrations was similar to, but lower than, that in the above-mentioned studies. Therefore it is possible that alcohol may have a direct effect on β -carotene disposition in humans, as in these animal studies.

TABLE 4

The paired difference between the alcohol phase and the alcohol-free phase in the adjusted mean plasma carotenoid, tocopherol, and retinol concentrations¹

	Difference	P
α -Carotene	3.1 \pm 1.4	0.027
β -Carotene	92.6 \pm 42.8	0.034
Cryptoxanthin	- 122.2 \pm 72.4	NS
Lutein/zeaxanthin	- 139.6 \pm 63.4	0.031
Anhydrolutein	10.6 \pm 15.8	NS
Lycopene	- 49.7 \pm 72.3	NS
Total carotenoids	- 127.7 \pm 209.1	NS
α -Tocopherol (μ mol/L)	- 8.1 \pm 11.6	NS
γ -Tocopherol (μ mol/L)	0.9 \pm 4.6	NS
Retinol (μ mol/L)	0.02 \pm 0.1	NS

¹ Adjusted for the average specific or total carotenoid intake and total energy intake.

² $\bar{x} \pm$ SEM; $n = 18$. A positive value refers to an increase with alcohol ingestion, whereas a negative value refers to a decrease with alcohol ingestion.

Alcohol intake increased plasma HDLC concentrations by 8% and reduced plasma LDLC concentrations by 11%. The direction and magnitude of these effects were similar to those in earlier studies (10, 22, 23). Although the hydrocarbon carotenoid β -carotene has been described as being primarily transported by LDLC, its ability to exchange lipoprotein carriers has also been reported (24). Because α - and β -carotene have similar geometric structure, the potential exchange capacity of β -carotene might also belong to α -carotene. Therefore, HDLC transport of plasma α - and β -carotene could have been enhanced when participants consumed alcohol, as compared with the alcohol-free state, and led to higher plasma carotenoid concentrations.

The direct association between alcohol and plasma β -carotene observed in this study was in contrast with earlier inverse associations reported in men (more so than in women) of healthy, free-living populations (8, 9). Men drink alcohol in higher quantities than do women. Indeed, too few women drank alcohol in any appreciable amount to examine the association between alcohol and plasma β -carotene (8, 9). Compared with men, women in the United States have a higher intake of specific and total carotenoids (25). Research on the distribution of the individual carotenoids on the lipoprotein fractions in cholesterol has been conducted in men (11), yet premenopausal women have lower LDLC and higher HDLC concentrations (10) and higher plasma α - and β -carotene concentrations than men of comparable age (26, 27). Sex differences in alcohol intake, dietary carotenoid intake, and plasma lipid and carotenoid concentrations in the free-living state might provide explanations for the findings of this study in contrast with those of prior cross-sectional research.

Plasma lutein/zeaxanthin concentrations were significantly lower with alcohol ingestion than in the alcohol-free phase. Alcohol is recognized as a source of lipid peroxidation in animals and humans (28). Anhydrolutein, an oxidative byproduct of lutein/zeaxanthin (29), was slightly higher in plasma after alcohol ingestion (by 10 nmol/L after adjustment for confounding factors). The slight increase in this metabolite may indicate that it has a short interval as an intermediate and is rapidly converted to another form. These findings have potential implications for the chemopreventive capacity of lutein/zeaxanthin and its metabolites under the influence of alcohol.

The average total dietary carotenoid intake was 5.7–6.0 mg/d during this controlled diet study. A representative sample of US women aged 19–50 y, who participated in the 1986 Continuing Food Survey of Individual Intake, consumed an estimated 6 mg total carotenoids/d (30). Thus, the total dietary carotenoid intake in this study was similar to the intake in the typical US diet of women of reproductive age.

The laboratory procedure used to measure plasma carotenoid concentrations provided data on 11 carotenoid peaks (19). To reduce the likelihood of chance findings, the statistical analysis was initially limited to the five major plasma carotenoid peaks, and three of the five showed significant differences by alcohol intake. Because plasma lutein/zeaxanthin concentrations differed by alcohol intake, the metabolite anhydrolutein was analyzed by using the ANOVA models. The remaining five minor peaks in the laboratory analysis (phytoene, phytofluene, neurosporene, and γ - and ζ -carotene) appeared in the array with lycopene, which did not differ by alcohol intake. These

five carotenoids are found in tomatoes and tomato products (similar to lycopene) or in apricots, cantelopes, and peaches, which were not fed to the participants. (Additional data on these five carotenoids can be provided on request.)

In summary, 18 nonsmoking, premenopausal women who participated in a controlled dietary study during three menstrual cycles in which they ingested 30 g alcohol/d or during three alcohol-free menstrual cycles experienced an increase in plasma α - and β -carotene concentrations and a decrease in plasma lutein/zeaxanthin concentrations with alcohol ingestion. The positive effect of alcohol on plasma α - and β -carotene concentrations was similar to the direct effect demonstrated in the controlled dietary study of baboons, whose ethanol intake was considerably higher. To further address the mechanisms underlying the relation between alcohol and plasma carotenoids, research efforts should focus on the interaction of alcohol intake and dietary sources of or pharmaceutical doses of specific carotenoids on plasma carotenoid and lipid concentrations in women and men under controlled dietary conditions. ■

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