Short Communication

Carotenoids and Menstrual Cycle Phase in Young Women"1

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Abstract

An association between serum carotenoid concentrations and risk for certain cancers has been observed in epidemiological studies. Determinants of serum carotenoid concentrations are known to include dietary intake, plasma lipid concentrations, and body mass. Menstrual cycle phase, which has not been adequately addressed in previous studies, has been suggested to be a possible additional factor to consider in the interpretation of these values in women. We evaluated hormonal status, serum carotenoids, cholesterol, and triglycerides in 48 healthy women at early follicular, mid-luteal, and late luteal phases of one menstrual cycle. Eating patterns were assessed with diet records at two 3-day intervals during the cycle. Analysis was focused on the 30 subjects who were determined to have ovulated during the menstrual cycle under observation. Serum cholesterol was significantly decreased (P < 0.05) in the late luteal phase of an ovulatory cycle. Lutein concentration was increased in the early follicular phase (P < 0.05) and α-carotene was increased in the mid-luteal phase (P < 0.05) only if uncorrected for total cholesterol. Other carotenoids did not vary across the menstrual cycle, whether corrected or uncorrected for total cholesterol concentration. In normal healthy ovulating women, serum carotenoids do not appear to vary with menstrual cycle phase when corrected for serum cholesterol concentrations.

Introduction

Carotenoids are currently of great interest in cancer chemoprevention because evidence suggests that they may exhibit multiple biological activities and anticarcinogenic effects (1–3). Surprisingly little is known about their basic metabolism and physiological effects, particularly in women. Increased knowledge in this area will help to refine the use of plasma or serum carotenoids as a biomarker for dietary intake in cohort studies and clinical trials (4). Previous reports from epidemiological and clinical studies have identified several determinants of circulating carotenoid concentrations, including dietary intake, body mass, smoking status, alcohol intake, and blood lipid concentrations (5–9). Changes in circulating lipids and lipoproteins appear to occur across the menstrual cycle (10); carotenoids in circulation are associated primarily with lipoproteins. Hence, it is possible that in women, menstrual cycle phase may also influence serum carotenoid concentrations. If so, this variable would influence the design and interpretation of investigations of the role of carotenoids in human health, particularly those relevant to health and disease in women. The only previously reported study of this relationship failed to determine the precise hormonal characteristics of the menstrual cycles of the subjects (11).

This cross-sectional study was conducted to describe the effect of menstrual cycle phase on serum carotenoid and cholesterol concentrations in normal, healthy, free living ovulatory women and to evaluate the relationship between serum carotenoid and cholesterol concentrations and hormonal status of young women.

Materials and Methods

Study Population. This investigation was part of a larger project examining the relationship between eating behavior and menstrual function in women. Forty-eight subjects were recruited from among the female undergraduate students of a major midwestern university. Inclusion criteria were a history of regular menstrual cycles, normal weight for height (body mass index between 18 and 25 kg/m²), and age between 18 and 20 years. In this report, we include all women (n = 30) who also fulfilled the following additional criteria: biochemical evidence of ovulation in the cycle under observation. To remove excessive exercise as a confounding variable, all subjects who reported exercising for >60 min/day, or >7 h/week were excluded. Other exclusion criteria included pregnancy, use of any oral medications (including oral contraceptives in the preceding 3 months), and any evidence of renal, hematological, cardiovascular, neurological, or hepatic disease. Each subject received a complete medical history and physical examination, and routine screening clinical laboratory tests were conducted. Procedures for this study were approved by the Institutional Review Board of the University of Michigan School of Medicine.

Procedures. Subjects were admitted to the CRC3 for a 24-h period at three intervals during one menstrual cycle (at approximate days 6, 21, and 28). While in the CRC, pulsatile secretory characteristics of hypothalamic-pituitary-gonadal axis function were measured, and results of the pulsatility studies will be presented elsewhere. A 12-h fasting

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1 The abbreviations used are: CRC, Clinical Research Center; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LH, luteinizing hormone; NIST, National Institutes of Standards and Technology.

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blood sample was obtained at 8 a.m. for measurement of serum carotenoids, cholesterol, and triglycerides. From the mid-follicular to late luteal phases of the cycle under study, subjects collected daily urine samples for measurement of LH concentration. Ovulatory function was determined on the basis of the hormonal measurements.

Body composition was estimated using caliper measurements (Lange, Cambridge, MD) of skinfold thickness and percentage body fat was calculated by the method of Durnin and Wormersley (12). Subjects were instructed to maintain their usual dietary patterns throughout the study.

At the first and second CRC visits (approximate menstrual cycle days 6 and 21), subjects were instructed to record all food and beverages consumed for a 3-day period during the subsequent days, including 2 weekdays and 1 weekend day, to reflect intake during the follicular and luteal phases of the menstrual cycle. Data obtained from these food records were first analyzed with a microcomputer dietary analysis system based on the United States Department of Agriculture food content data, supplemented with manufacturer information, and processed with the Nutritionist III interface software (N-Squared Computing, Salem, OR). Dietary intake of carotenoids was computed from the diet records using the recently-released United States Department of Agriculture-National Cancer Institute carotenoid food composition data base, which contains values for α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein plus zeaxanthin in >2400 fruits and vegetables and multingredient foods containing fruits and vegetables (13, 14). The two sets of 3-day records were used to compare subject intake during follicular and luteal phases.

**Serum Carotenoid Analysis.** Serum carotenoids were separated and quantified based on the HPLC method of Bieri et al. (15), as modified by Craft et al. (16, 17), with further modifications to reduce oxidative loss and improve recovery of compounds during analysis. Following collection by venipuncture, blood was allowed to clot and was separated with refrigerated centrifugation at 2300 × g for 10 min to obtain sera. Samples were stored at −70°C until lipid extraction and HPLC analysis, which was conducted with a Varian Star 9010, 9050 system with variable wavelength UV/VIS detector (Varian Analytical Instruments, Walnut Creek, CA) with the wavelength set at 450 nm. The mobile phase was acetonitrile:methanol:methylene chloride (70: 10:20) with triethylamine (0.13%) (v/v) and ammonium acetate (0.01%) (w/v) modifiers used to enhance recovery. The column used was a Supelco (Bellefonte, PA) Supelcosil LC-18 (25 cm × 4.6 mm × 5 µm). This analytical method measures 90% of the total plasma carotenoids present and permits quantification of the predominant serum carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein plus zeaxanthin). With this acetonitrile-based method, the peak designated lutein is assumed to also contain the isomerically related carotenoid, zeaxanthin. Accuracy was assessed by periodic analysis of NIST Standard Reference Material SRM 986, fat-soluble vitamins, and a pooled-plasma reference sample was analyzed concurrent with batches of study samples to monitor analytical precision. Values for carotenoid concentrations were within 10% of NIST values with this analytic method, and day-to-day coefficients of variation during analysis of study samples were <5%.

**Serum Lipid Analysis.** Determinations of serum cholesterol and triglycerides were performed with the Kodak Ektachem Analyzer system (Eastman Kodak Co., Rochester, NY) (18). Standard reference materials from the manufacturer were used to validate analytical precision of these procedures. Cholesterol values were also used in the evaluation of carotenoid data, corrected as a ratio of carotenoid/cholesterol concentration, the approach applied to tocopherols as described by Bieri et al. (19, 20).

**Analysis of Hormonal Status.** On each of the scheduled test days (approximate days 6, 21, and 28 of the menstrual cycle), a blood sample obtained at 8 a.m. was used to measure serum estradiol and progesterone concentrations. Estradiol and progesterone were quantified with enzyme-linked immunosorbent assays, using the Boehringer Mannheim Automated ES 300 Immunoassay system (Indianapolis, IN). The CIBA-Corning ACS 180 LH Chemilumimetric Immunoassay (Pittsburgh, PA) was used to quantify serum and urinary LH. With this ES 300 system, intraassay coefficient of variation for estradiol was 4.5% and for progesterone it was 2.1%. Intraassay coefficient of variation for LH using the ACS 180 system was 2.2%.

**Statistical Analysis.** Because many of the variables were not normally distributed, all analyses were conducted using nonparametric methods. Friedman’s two-way analysis of variance was used to compare the mean levels of a variable at all three times. When Friedman’s test is significant, the two most dissimilar groups are not equal; then pairwise comparisons are used to test the level of the intermediate group to each of the extreme groups. To compare the mean levels of a variable at two time points, the Wilcoxon rank sum test was applied to the differences between the times. Spearman rank correlations were computed between pairs of variables at a single time point. All tests are two-sided. For descriptive purposes means ± SEMs are reported. All analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC).

**Results**

**Study Subjects.** All 48 subjects completed the study protocol. However, for 14 of the subjects incomplete data from urinary samples made it impossible to determine whether ovulation occurred during the menstrual cycle. Also, four subjects were determined to have been anovulatory during the menstrual cycle. Thus, a total of 30 subjects were determined to have ovulated during the menstrual cycle under observation, based on hormonal data including a urinary surge in LH concentration, and were eligible for this study. This sample size is comparable with the number of subjects used in previous studies of variability in plasma lipid and micronutrient concentrations over the menstrual cycle (10, 11).

Body mass index of the study subjects (n = 30) was 22.13 ± 0.35 kg/m² and percentage body fat was 24.58 ± 0.88.

**Effect of Menstrual Cycle Phase.** The pattern of variation of serum cholesterol and carotenoids that was observed over the three time points during the menstrual cycle is illustrated in Fig. 1. As indicated in the figure, serum cholesterol was lower in the late luteal phase (P < 0.05), serum lutein was higher in the early follicular phase (P < 0.05), and serum α-carotene was higher in the mid-luteal phase (P < 0.05). However, when carotenoid concentrations were corrected for total serum cholesterol as described, the differences in lutein and α-carotene concentrations were not significant (P = 0.23 and 0.27, respectively). Other carotenoids did not vary significantly across the menstrual cycle.
Fig. 1. Serum concentrations of cholesterol and carotenoids at three phases of an ovulatory menstrual cycle (early follicular, mid-luteal, and late luteal). Columns, mean; bars, SEM; n = 30. * significantly lower than the other two phases at late luteal for cholesterol (P < 0.05); significantly higher than the other two phases at early follicular for lutein (P < 0.05); and significantly higher than the other two phases at mid-luteal for α-carotene (P < 0.05).

whether corrected or uncorrected for cholesterol concentration.

Serum triglyceride concentrations across the menstrual cycle were also not statistically different, as shown in Table 1, which summarizes descriptive data. As expected, serum estradiol and progesterone concentrations were lower in the follicular phase (P < 0.0001 and P < 0.0001, respectively) than in the other phases.

Dietary Intake. Qualitatively, the diets of the majority of these subjects tended to consist of many convenience and snack food items and none reported use of β-carotene supplements. Table 2 summarizes dietary intake data computed from the food records collected in the follicular and luteal menstrual cycle phases. Significant differences in intake of energy, macronutrients, cholesterol, and total carotenoids were not observed when results from the follicular and luteal phases were compared. However, lutein intake was higher in the follicular phase (P < 0.05), and a trend toward higher β-carotene intake during the follicular phase (P = 0.07) was also observed. Significant correlations between serum and dietary carotenoids were not found.

Relationship between Carotenoids, Cholesterol, and Hormone Concentrations. Correlations between nutritional variables (serum carotenoids, serum cholesterol, and di-
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However, menstrual cycle phase was observed to have women when corrected for serum cholesterol concentration, confirming results from previous investigations. A notable effect on total serum cholesterol concentration, serum camotenoid concentrations in normal healthy young and progesterone concentrations. In the present study, menstrual cycle phase on serum carotenoids in normal healthy young.

To our knowledge, this is the first reported investigation of which hormonal status and ovulatory function were assessed. Menstrual cycle phase in an ovulatory cycle did not influence serum carotenoid concentrations in normal healthy young women when corrected for serum cholesterol concentration. However, menstrual cycle phase was observed to have a notable effect on total serum cholesterol concentration, confirming results from previous investigations.

The possibility that menstrual cycle phase could influence serum carotenoid concentrations in women is suggested by fluctuations of the lipoproteins, with which carotenoids are associated in the circulation (21). As recently reviewed by Krummel et al. (10), fluctuations in total cholesterol concentration have been previously shown to occur throughout the menstrual cycle, with the lowest level likely to occur in the late luteal phase (as observed in the present study) or in the menstrual phase. Mattsson et al. (22), who followed 22 normally menstruating women during one menstrual cycle, reported a significantly higher LDL:HDL ratio in the follicular phase than in the other phases. Lipoprotein transport of specific carotenoids in women, the profile of which circulating lipoproteins differs from that in men, has not yet been established. In men, 55% of the carotenoids are associated with LDL, with HDL carrying 31%, and the remaining associated with other lipoprotein fractions (21).

In a single previous report on the effect of menstrual cycle phase on serum carotenoids, Tangney et al. (11) evaluated concentrations in blood samples from nine women at five separate times each month for 2 months. The women were determined to be eumenorheic based on subject history of regular menstrual cycles in the preceding 6 months, and total serum cholesterol, triglycerides, estradiol, and beta-carotene were quantified. Without any determination of ovulatory status, menstrual cycle phase was assumed on the basis of circulating estradiol concentrations. They concluded that plasma beta-carotene was 33% higher during the follicular phase (days 7–10) than during the menstrual phase (days 1–5) after adjusting for plasma cholesterol concentration. In contrast, fluctuations in carotenoid concentrations between the time points measured in the present study (approximate days 6, 21, and 28) were not evident when corrected for cholesterol concentrations. Compared to the study by Tangney et al. (11), an important difference in the present study is that carotenoid status was evaluated in women for whom hormonal and ovulatory status was established with biochemical methods, which may have influenced the outcome and conclusions due to improved precision. The subjects in the present study were better defined and perhaps a more homogeneous group. Also, the measurements were obtained at different time intervals across the menstrual cycle in these two studies, although hormonal influences would be expected to be similarly reflected in these intervals.

No previous study has involved measurement of carotenoids other than beta-carotene in the evaluation of menstrual cycle phase effect. Lutein and beta-cryptoxanthin are oxygenated carotenoids (i.e., beta-cryptoxanthin has one hydroxy group and lutein contains two), and the others that were measured are hydrocarbons. When uncorrected for serum cholesterol, some variability in lutein and alpha-carotene concentrations was observed in the present study. In particular, the pattern of variation in serum lutein concentration appears different from the others (Fig. 1). This may relate in part to variability in dietary intake of this carotenoid, which is discussed below. Also, absorption and plasma clearance rates of various carotenoids and their isomers appear to differ, based on results from a few kinetic studies (23). Lipoprotein transport differences may also affect the pattern observed in the circulating pool. In men, Clevendie and Bier (21) found the hydrocarbon carotenoids predominantly with LDL, beta-cryptoxanthin to be equally distributed between LDL and HDL, and lutein and zeaxanthin to be associated primarily with HDL (21).

Although the total circulating concentrations appear to remain relatively stable across the menstrual cycle, the

### Table 1

<table>
<thead>
<tr>
<th>Serum concentrations of cholesterol, triglycerides, carotenoids and hormones, by menstrual cycle phase, for the study subjects *</th>
<th>Early follicular</th>
<th>Mid-luteal</th>
<th>Late luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/liter)</td>
<td>3.837 ± 0.131</td>
<td>3.962 ± 0.153</td>
<td>3.645 ± 0.139</td>
</tr>
<tr>
<td>Triglycerides (mmol/liter)</td>
<td>0.89 ± 0.076</td>
<td>0.787 ± 0.048</td>
<td>0.732 ± 0.069</td>
</tr>
<tr>
<td>Lutein (μmol/liter)</td>
<td>0.443 ± 0.032</td>
<td>0.375 ± 0.031</td>
<td>0.370 ± 0.027</td>
</tr>
<tr>
<td>beta-Cryptoxanthin (μmol/liter)</td>
<td>0.184 ± 0.014</td>
<td>0.193 ± 0.019</td>
<td>0.172 ± 0.012</td>
</tr>
<tr>
<td>Lycopene (μmol/liter)</td>
<td>0.706 ± 0.060</td>
<td>0.740 ± 0.051</td>
<td>0.653 ± 0.045</td>
</tr>
<tr>
<td>alpha-Carotene (μmol/liter)</td>
<td>0.068 ± 0.011</td>
<td>0.078 ± 0.012</td>
<td>0.067 ± 0.010</td>
</tr>
<tr>
<td>beta-Carotene (μmol/liter)</td>
<td>0.305 ± 0.033</td>
<td>0.314 ± 0.032</td>
<td>0.287 ± 0.028</td>
</tr>
<tr>
<td>Estradiol (pmol/liter)</td>
<td>131.5 ± 13.2</td>
<td>386.1 ± 34.7</td>
<td>359.1 ± 36.9</td>
</tr>
<tr>
<td>Progesterone (μmol/liter)</td>
<td>0.623 ± 0.541</td>
<td>14.498 ± 2.859</td>
<td>16.142 ± 2.547</td>
</tr>
</tbody>
</table>

* Mean ± SEM; n = 30.

### Table 2

<table>
<thead>
<tr>
<th>Daily intakes of selected dietary components, in two phases of the menstrual cycle, for the study subjects *</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>7517 ± 453</td>
<td>8000 ± 547</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>64.9 ± 7.0</td>
<td>62.5 ± 3.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>245.2 ± 15.5</td>
<td>273.8 ± 25.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>64.5 ± 5.9</td>
<td>66.2 ± 5.3</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>9.3 ± 0.9</td>
<td>10.3 ± 0.9</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>213.3 ± 33.4</td>
<td>181.3 ± 14.0</td>
</tr>
<tr>
<td>alpha-Carotene (μg)</td>
<td>324 ± 72</td>
<td>332 ± 106</td>
</tr>
<tr>
<td>beta-Carotene (μg)</td>
<td>2132 ± 424</td>
<td>1706 ± 430</td>
</tr>
<tr>
<td>beta-Cryptoxanthin (μg)</td>
<td>1 ± 1</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>Lycopene (μg)</td>
<td>1524 ± 227</td>
<td>1834 ± 390</td>
</tr>
<tr>
<td>Lutein (μg)</td>
<td>1687 ± 322</td>
<td>1189 ± 413</td>
</tr>
<tr>
<td>Total carotenoids (μg)</td>
<td>5679 ± 678</td>
<td>5068 ± 975</td>
</tr>
</tbody>
</table>

* Mean ± SEM; n = 30.

* Trend toward being higher than in other menstrual cycle phase (P > 0.05).

* Significantly higher than in other menstrual cycle phase (P < 0.001).
distribution of the carotenoids among the lipoproteins may vary with phase of the cycle. An increase in cholesterol-rich HDL and a decline in LDL has been demonstrated to occur during the luteal phase (10, 22), and HDL may be an important carrier of carotenoids at physiological levels in women. As suggested by Johnson and Russell (24), who examined the distribution of β-carotene among lipoproteins following a large p. o. dose, transfer of carotenoids among the lipoproteins may occur. One limitation of the present study is that carotenoid concentrations of the lipoprotein fractions were not determined.

The carotenoids appear to be relatively unregulated in the circulation (6) where they are associated with fluctuating cholesterol-rich lipoproteins. A closer examination of the relationship between circulating carotenoids and the lipoproteins provides some insight to explain why menstrual cycle phase appears to exert little effect. As mentioned above, carotenoids can be associated with a variety of lipoproteins and are not specifically limited to LDL. Also, the volume of lipoproteins in circulation far exceeds the amount of carotenoids. As described by Esterbauer et al. (25), carotenoids comprise a small fraction of the antioxidant micronutrients associated with LDL when evaluated on a molar basis, particularly when compared with α-tocopherol. Thus, fluctuations in lipoprotein concentrations would have to be substantial before a significant effect on total serum carotenoid concentrations is likely to be evident. In support of this concept, a correlation between serum or plasma lipids and carotenoids is observed in large population-based studies in which a wide range of lipid values occur (5, 7, 9), and is not observed in studies involving smaller or more homogeneous groups with a narrower range of lipoprotein and total lipid concentrations (6, 26).

Serum concentrations of the carotenoids measured in the present study were within the normal ranges reported previously (15, 25). Although the mean β-carotene concentration was lower than that observed in older populations who report more varied diets and supplement usage (7), these serum values were comparable to those recently reported for larger populations of premenopausal women (27). It is possible that in women who have elevated serum carotenoids (e.g., associated with the use of β-carotene supplements), a more notable change in circulating concentrations might occur during the menstrual cycle.

Mean dietary carotenoid intake of the subjects in the present study was in the range (but below the mean) of values reported in studies of larger groups of premenstrual women (27), which is reflective of a diet low in fruits and vegetables (4). Differences in the intake of macronutrients and total carotenoids across the menstrual cycle were not observed, although the small sample size and limitations in dietary assessment methodology are weaknesses. In the present study, lutein intake was observed to be higher in the follicular phase than in the luteal phase, and this variability may have contributed to the observed pattern in serum lutein concentration.

These carotenoid intake data should be cautiously interpreted, because the number of days of food records necessary to accurately describe vitamin A or carotenoid intake is likely to be greater than six, as was used in the present study (28). High day-to-day variability is characteristic of carotenoid intake, which adversely affects the accuracy of using diet records in dietary assessment (27, 28). In the present study, significant correlations between dietary intake and serum concentrations were not observed. As reported by Forman et al. (8, 27, 28), correlations between carotenoid intake and plasma concentrations are modest even in larger groups of subjects and when using methods designed to optimize estimations of intake. This incongruity is often evident in studies of cancer risk; e.g., Potischman et al. (29) found plasma concentrations (but not dietary levels) of β-carotene to be associated with reduced risk for breast cancer.

In conclusion, serum carotenoid concentrations do not vary significantly across an ovulatory menstrual cycle when corrected for serum cholesterol. Independent hormonal influences on circulating carotenoids are unlikely to preclude the usefulness of these biomarkers in epidemiological studies and clinical trials when serum lipids are also examined and used in the interpretation of these data.

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