

# The fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle: a controlled diet study<sup>1,2</sup>

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See corresponding editorial on page 640.

**ABSTRACT** This is the first controlled diet study to examine the fluctuation of plasma carotenoids, lipoproteins, and serum hormone concentrations by phase of the menstrual cycle. Non-smoking, premenopausal women ( $n = 12$ ) with confirmed ovulatory cycles were given a standard diet with 10 mg total carotenoids/d for two cycles under isoenergetic conditions. Blood was drawn for simultaneous measurement of carotenoids, lipoproteins, and hormones on menses days 1–2, 4–6, 11 through 1 d after the luteinizing hormone surge, and 7–8 d after the surge, representing the menses, early and late follicular, and midluteal phases, respectively. Regression modeling with adjustment for plasma cholesterol concentrations was used to compare mean individual and total plasma carotenoid concentrations by phase of the cycle. Plasma carotenoid concentrations were at their lowest at menses and significantly higher thereafter, except for  $\alpha$ -carotene. Compared with plasma concentrations at menses,  $\beta$ -carotene peaked (increased by 9%,  $P = 0.01$ ) in the late follicular phase. Plasma lutein/zeaxanthin and anhydrolutein concentrations were higher by 8–11% ( $P \leq 0.006$ ) and by 15–31% ( $P \leq 0.02$ ), respectively, during the last three phases. Plasma lycopene and phytofluene concentrations peaked (increased by 12%,  $P = 0.004$ ; and by 21%,  $P = 0.006$ , respectively) at the midluteal phase. This cyclic fluctuation may affect the estimation of the plasma carotenoid-disease relation in studies of premenopausal women. *Am J Clin Nutr* 1996;64:559–65.

**KEY WORDS** Menstrual cycle, carotenoids, lipoproteins, controlled diet

## INTRODUCTION

Recent epidemiologic evidence suggests a modest inverse association between  $\beta$ -carotene intake and breast cancer risk (1, 2). Experimental studies document the ability of specific carotenoids and vitamin A to inhibit mammary carcinogenesis in rats (3, 4). Carotenoids exhibit chemopreventive capabilities by neutralizing free radicals and reactive oxygen species during normal cellular metabolism and oxidative stress, and consequently protect against nuclear damage (5–12). Moreover, a subgroup is enzymatically converted to retinol and its metabolites, which have recognized anticarcinogenic capabilities (5–12).

Plasma carotenoids are transported by specific lipoprotein fractions. Plasma concentrations of  $\alpha$ - and  $\beta$ -carotene and

lycopene are primarily transported by the low-density lipoproteins (LDLs), whereas lutein/zeaxanthin is more evenly distributed between the high-density lipoproteins (HDLs) and LDL (13, 14).

Plasma lipoprotein concentrations fluctuate during the menstrual cycle (15–17). According to studies with frequent blood draws for hormone measurements to accurately determine the phase of the menstrual cycle, plasma LDL-cholesterol concentrations peak in the early follicular phase and plasma HDL-cholesterol concentrations peak in the late follicular phase (15–17).

Given the cyclic fluctuation of lipoproteins that transport the individual carotenoids, it is expected that carotenoids would also fluctuate by phase of the menstrual cycle. Yet, prior studies do not report any fluctuation of the carotenoids during the menstrual cycle (18, 19). Methodologic issues may have reduced the ability of the researchers to show any fluctuation, including relying on subject-reported date of last menses to estimate blood sampling schedules for serum hormone determinations and using food diaries at select intervals to estimate dietary carotenoid intake.

A study of the fluctuation of plasma carotenoids, lipoproteins, and serum hormones by phase of the menstrual cycle [Carotenoids, Lipids, Hormone Study (CLH)] was conducted in 20 healthy premenopausal women for three menstrual cycles in 1992. The purposes of each study cycle were the following: in cycle 1, each woman had a confirmed ovulatory cycle in the free-living state; in cycle 2, women were placed on a standard, daily, carotenoid-rich diet under controlled diet conditions; and in cycle 3, the cyclic pattern of plasma carotenoid concentrations was examined under the same controlled diet conditions as in cycle 2. This study asked: Do plasma carotenoids fluctuate?

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ate with plasma lipoprotein and serum hormone concentrations by phase of the menstrual cycle under controlled dietary conditions?

## SUBJECTS AND METHODS

### Subjects

Female volunteers were recruited by advertisements in communities surrounding the Beltsville, MD, area or were contacted from lists of participants of previous studies at the US Department of Agriculture Beltsville Human Nutrition Research Center (USDA-BHNRC). Respondents to the advertisements were initially screened by phone. Potential candidates were seen at the National Institutes of Health (NIH) Clinical Center for biochemistry tests, physical and gynecologic exams, and an in-person interview. Twenty women met the following eligibility criteria: 1) aged 20–34 y; 2) did not smoke; 3) had no history of chronic disease; 4) were not pregnant or taking oral contraceptives or other hormone preparations, or breast-feeding in the past 6 mo; 5) had no reported history or clinical signs of gynecologic problems, with reported menstrual cycle length ranging from 26–32 d; 6) were within 20% of weight-for-height based on age and sex-specific referent values in the 1993 Metropolitan Life Insurance Tables (20); 7) had biochemistry test results within the normal range for plasma cholesterol, triacylglycerol, and hemoglobin concentrations; 8) were not following a restricted diet or regularly using vitamin-mineral supplements and were willing to stop supplementation during the study; and 9) either abstained from alcohol or drank < 6 drinks/wk and < 2 drinks/d, and were willing to abstain from alcohol for the controlled diet study (21). All women signed an informed consent form. The protocol was approved by the institutional review boards of the George Washington University School of Medicine, the National Cancer Institute, and the US Department of Agriculture.

Of the 20 women who began the free-living study, one dropped out, one had an anovulatory cycle, and two left for medical reasons that were unrelated to the study. Two others completed the free-living cycle portion, but decided not to participate in the controlled diet study. Therefore, 14 women began the controlled diet study. However, two were removed from the data analysis because one experienced an anovulatory cycle and the other did not show a plasma response to the carotenoid-rich diet, leaving 12 with data for the analysis of all three menstrual cycles.

### Study design

The study was designed as a free-living study for one menstrual cycle followed by a controlled diet study for two cycles. Before the free-living study, participants were trained in, viewed an instructional video about, and had to demonstrate the ability to complete food records using premeasured cooking utensils (22), and were familiarized with the menstrual day sequence of blood draws (by MF). Each participant began the free-living study on her first menses day in March–April 1992. Food records were reviewed daily for the following: food labels and recipes, portion sizes, identification of the time and place where each food was consumed, and an indication whether food intake on that day reflected her usual intake. After form review, participants were contacted in-person or by

phone for any items of clarification about the previous day's intake. A daily log was kept by the field coordinator (PM) to record the collection of food records, blood specimens, hormone results, phase of the menstrual cycle, the woman's health status, and any use of medications. Food records were submitted to the Nutrition Coordinating Center of the University of Minnesota for calculation of nutrient intake, including individual and total carotenoids based on the USDA-NCI Carotenoid Food Composition Database (23, 24).

The controlled diet study was conducted at the USDA-BHNRC and consisted of a 7-d repeated menu cycle. The daily intake of energy was distributed as 36% from fat, 19% from protein, and 55% from carbohydrates with 3.4 g fiber/100 kJ and a ratio of polyunsaturated to saturated fatty acids (P:S) of 0.53. Foods from each day's menu were homogenized and analyzed for macronutrients twice during the study. A standard set of carotenoid-rich foods (peas, carrots, broccoli, and tomato sauce) were given daily in the same amount at dinnertime. These foods were identified as the major sources of  $\beta$ -carotene, lutein, and lycopene intakes in women of reproductive age in the United States (25). The carotenoid-rich food sources were analyzed for specific carotenoid content at the Nutrient Composition Laboratory (NCL) of the USDA-BHNRC. They provided 0.7 mg  $\alpha$ -carotene/d, 2.5 mg  $\beta$ -carotene/d, 2.6 mg lutein/d, and 4.3 mg lycopene/d, for a total of  $\approx$ 10 mg carotenoids/d. No other carotenoid-rich foods were eaten during the controlled diet study. All meals were prepared at the BHNRC. The women ate their meals at the BHNRC dining facility, except for carryout lunches on weekdays and carryout meals on weekends and holidays.

To maintain isoenergetic conditions, participants were weighed daily before breakfast. If a woman lost or gained  $\geq$  1 kg and maintained that weight for  $\geq$  3 d, her energy intake was increased or decreased in increments of 47.6 kJ (200 kcal). Vitamin and mineral supplements and alcohol were prohibited throughout the two controlled diet cycles of the study.

### Measurement of plasma carotenoids, lipids, and serum hormones

Blood samples were collected between 0600 and 0700 from women who had fasted for > 10 h. Samples were collected in EDTA-containing and EDTA-free evacuated glass containers for plasma carotenoid and lipoprotein and serum hormone analyses, respectively. Blood draws occurred on selected days of the menstrual cycle (Table 1). Blood was drawn during the menses (menses days 1–2); menses day 1 was defined as the first day of the menstrual period with a full blood flow. Blood draws for the early follicular phase were on menses days 4–6.

**TABLE 1**  
Blood specimen collection schedule: Carotenoids, Lipids, Hormone Study<sup>1</sup>

Phase of the cycle	Menses days	Laboratory determinations
Menses	1–2	E <sub>2</sub> , carotenoids, lipoproteins
Early follicular	4–6	E <sub>2</sub> , carotenoids, lipoproteins
Late follicular	11-LH surge + 1 d	E <sub>2</sub> , LH, P <sub>4</sub> , carotenoids, lipoproteins
Midluteal	7–8 d after LH surge	E <sub>2</sub> , P <sub>4</sub> , carotenoids, lipoproteins

<sup>1</sup> E<sub>2</sub>, estradiol; LH, luteinizing hormone; P<sub>4</sub>, progesterone.

The late follicular phase included menses days 11 through 1 d after the luteinizing hormone surge. An ovulatory cycle was defined as having a serum luteinizing hormone concentration  $> 30$  IU/L at the luteinizing hormone surge and a serum progesterone concentration between 32 and 64 nmol/L at the midluteal phase. The Ovuquick urine test (Quidel, San Diego) to determine ovulation was routinely used during the late follicular phase as a back up to the blood hormone collection and replaced serum hormone analyses for one participant who became ill and was unable to have her blood drawn. The midluteal phase was defined as the 7–8 d after the luteinizing hormone surge.

Hormone determinations were performed by the Immuno-assay Laboratory of the Genetics and In Vitro Fertilization Institute, Fairfax, VA, on the same day as the blood draw. Serum estradiol and progesterone concentrations were measured by direct radioimmunoassay (Diagnostic Products Corp, La Jolla, CA). Serum luteinizing hormone was quantified by the monoclonal Immuno-Radio-Metric-Assay (IRMA; Serono, Randolph, MA). Procedures followed the analytical methods described by Munabi et al (26). Intra- and interassay CVs for hormone analysis were  $< 7.0\%$  and  $< 8.1\%$ , respectively, for estradiol;  $< 5.8\%$  and  $< 11.7\%$ , respectively, for progesterone; and  $< 11.4\%$  and  $< 13.1\%$ , respectively, for luteinizing hormone. Hormone values were transmitted to the principle investigator daily to determine whether a woman had completed one phase of the cycle and could stop blood specimen collection until the beginning of the next phase.

Lipoprotein fractionations and analyses were performed by the George Washington University Lipid Research Clinic Laboratory (LRC-Washington DC). HDL-cholesterol fractions were isolated on the day of the blood draw by using the precipitation procedure of Gidez et al (27) as described previously (28). The supernatant fractions were promptly placed at  $-70^{\circ}\text{C}$  in Nunc polypropylene vials (Nunc Inc, Naperville, IL). To avoid day-to-day analytic variations, all samples from an individual were batched and analyzed sequentially as a set. Cholesterol and triacylglycerols were determined enzymatically (28). Control procedures followed the protocol for the LRC Program (29). The CVs for analysis of total cholesterol and triacylglycerol control pools averaged 1.5% and 1.8%, respectively. LDL-cholesterol concentrations were derived by using the equation of Friedewald et al (30). The laboratory maintains standardization with the Centers for Disease Control and Prevention (CDC) for analysis of cholesterol, triacylglycerols, and HDL cholesterol.

Plasma carotenoid analyses were performed by the NCI-USDA-BHNRC (Beltsville, MD). Blood samples were immediately centrifuged at  $1500 \times g$  for 20 min at  $4^{\circ}\text{C}$ , and plasma aliquots were then stored in polypropylene vials at  $-70^{\circ}\text{C}$  until the end of the study. To minimize day-to-day variation, all specimens of an individual for each menstrual cycle were batched with quality control pools at the beginning and end of the batch. Individual daily plasma  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein/zeaxanthin, anhydrolutein, lycopene, phytoene, phytoluenene, and retinol concentrations were analyzed by HPLC using a  $\text{C}_{18}$  reversed-phase column as described by Khachik et al (31). Pure forms of the carotenoids were used as reference standards for quantitation. The laboratory maintains standardization by participating in the National Institute of Standards and Technology Fat Soluble Vitamin

Round Robin Program. The CVs for analysis of  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin, and lycopene in control pools averaged 9.6%, 8.8%, 5.9%, and 9.5%, respectively.

### Statistical analysis

In the initial analysis, measures of central tendency for plasma concentrations of specific and total carotenoids, retinol, cholesterol, LDL and HDL cholesterol, and serum hormones were calculated for each phase of the menstrual cycle and each cycle: 1) the free-living cycle; 2) CDI, the first controlled diet menstrual cycle, and 3) CDII, the second controlled diet menstrual cycle. Plasma carotenoid concentrations of each subject were averaged over 2–3 consecutive days in the menses, early follicular, and midluteal phases, respectively. In the late follicular phase, measurements on 2 d before plus the day of the luteinizing hormone surge were averaged to estimate plasma carotenoid concentrations. Daily plasma lipoprotein concentrations were matched with plasma carotenoid concentrations by phase of the menstrual cycle.

After the initial analysis, regression modeling with categorical variables as predictors was used to compare mean individual and total plasma carotenoid concentrations by phase of the menstrual cycle in the CDII [SAS, PROC GLM (SAS Institute, Cary, NC)]. The regression models included a categorical variable (ie, a separate intercept) for each person to take into account the correlation among repeated measurements over the cycle for each person. An advantage of this regression-modeling statistic was its ability to use different multiples of consecutive days of plasma concentrations for each person because the number of blood draw days varied by phase. The least square means ( $\pm$  SE) for each carotenoid by phase of the menstrual cycle were generated from the models. Subsequent models included an adjustment for plasma concentrations of total, LDL, and HDL cholesterol. All findings were considered significant at a  $P$  value  $< 0.05$ .

## RESULTS

The women who participated during all three menstrual cycles of the study were, on average, 27 y old, had a high school education, and were slightly thinner than reported recently for US women of reproductive age (32). Their average menstrual cycle length (for each study cycle) was 27 d (Table 2). Serum hormone concentrations during the three cycles were typical of women with ovulatory cycles (Table 3). Compared with the early follicular phase, serum estradiol concentrations were six- to sevenfold higher in the late follicular (luteinizing hormone surge) and 2.5- to 4-fold higher at the midluteal phase.

TABLE 2

Characteristics of the study participants: Carotenoids, Lipids, Hormone Study<sup>1</sup>

Age (y)	27 $\pm$ 3
Height (cm)	166 $\pm$ 5
Weight (kg)	58 $\pm$ 6
BMI (kg/m <sup>2</sup> )	21 $\pm$ 2
Education (y)	13 $\pm$ 2
Cycle length (d)	27 $\pm$ 2

<sup>1</sup>  $\bar{x} \pm$  SD;  $n = 12$ .

TABLE 3

Serum hormone concentrations during the free-living and the controlled diet cycles: Carotenoid, Lipids, Hormones Study<sup>1</sup>

Study cycle	Phase of the menstrual cycle			
	Menses	Early follicular	Late follicular	Luteal
Free-living				
Estradiol (pmol/L)	106 ± 40	117 ± 51	833 ± 202 <sup>2</sup>	580 ± 275
Luteinizing hormone (IU/L)			65 ± 32 <sup>2</sup>	
Progesterone (nmol/L)				48 ± 22
Controlled diet cycle I				
Estradiol (pmol/L)	92 ± 22	128 ± 29	976 ± 389 <sup>2</sup>	481 ± 217
Luteinizing hormone (IU/L)			76 ± 32 <sup>2</sup>	
Progesterone (nmol/L)				51 ± 16
Controlled diet cycle II				
Estradiol (pmol/L)	114 ± 33	110 ± 29	885 ± 323 <sup>2</sup>	389 ± 103
Luteinizing hormone (IU/L)			81 ± 38 <sup>2</sup>	
Progesterone (nmol/L)				48 ± 16

<sup>1</sup>  $\bar{x} \pm SD$ ;  $n = 12$ .<sup>2</sup> Serum estradiol and luteinizing hormone concentrations in the late follicular phase are the average of the measurements on the 2 d before and the day of the luteinizing hormone surge.

The average total dietary carotenoid intake for the free-living cycle was 6.3 mg/d, with the largest contributions being from  $\beta$ -carotene (33%), lutein/zeaxanthin (32%), and lycopene (29%) (Table 4). The range in the average total dietary carotenoid intake revealed more than a threefold difference in intake, with the lowest intake 3.2 mg/d and the highest intake 10.9 mg/d. During the midluteal phase of the free-living cycle, the individual carotenoids that made the largest contribution to total plasma carotenoid concentrations were as follows: lycopene (27%), lutein/zeaxanthin (19%), cryptoxanthin (19%), and  $\beta$ -carotene (18%) (Table 5).

Because of the large range in dietary carotenoid intake in the free-living cycle, participants were given 10 mg total carotenoids/d in the form of carotenoid-rich foods for two subsequent menstrual cycles (CDI and CDII). To evaluate the effects of the carotenoid-rich controlled diet on plasma carotenoid concentrations, we compared concentrations at the midluteal phase of the free-living and CDI cycles, because these were the last blood draw days reflective of the free-living diet and the first 20 or more days on the CDI cycle (Table 5). The average total plasma carotenoid concentrations had increased by 42%, with the largest increase in lycopene (by 66%) followed by  $\beta$ -carotene and lutein/zeaxanthin (by 40% each). All plasma carotenoid concentrations at menses in the CDII cycle were 1 SE

from the mean at the midluteal phase of the CDI, indicating that plasma carotenoid concentrations were near steady state (Table 5 and Table 6).

In the CDII cycle, individual and total plasma carotenoid concentrations were at their lowest at menses and increased during subsequent phases, except for  $\alpha$ -carotene, which remained constant throughout the cycle (Table 6). Mean plasma  $\beta$ -carotene concentrations were significantly higher (by 9%) during the late follicular phase than during the menses and early follicular phases ( $P \leq 0.01$ ). Concentrations of plasma lutein/zeaxanthin and its metabolite, anhydrolutein, were significantly higher (by 8–11% and by 15–31%, respectively) during all three phases after menses ( $P \leq 0.006$  for lutein/zeaxanthin and  $P \leq 0.02$  for anhydrolutein). In addition, plasma anhydrolutein concentrations were significantly higher during the midluteal than during the late follicular phase ( $P = 0.02$ ). Compared with plasma concentrations at menses, lycopene and phytofluene were significantly higher by 12% ( $P =$

TABLE 4

Specific and total dietary carotenoid intake during the free-living cycle compared with the two controlled diet cycles: Carotenoids, Lipids, Hormone Study<sup>1</sup>

Carotenoid intake	Free-living cycle	Controlled diet cycles <sup>2</sup>
	<i>mg/d</i>	
$\alpha$ -Carotene	0.32 ± 0.2	0.70
$\beta$ -Carotene	2.10 ± 0.9	2.50
Cryptoxanthin	0.60 ± 0.04	—
Lutein/zeaxanthin	2.00 ± 1.4	2.60
Lycopene	1.80 ± 0.8	4.30
Total carotenoids	6.30 ± 2.3	10.00

<sup>1</sup>  $\bar{x} \pm SD$ ;  $n = 12$ .<sup>2</sup> Means are presented based on an analysis of two batches of vegetables.

TABLE 5

Specific and total plasma carotenoid and retinol concentrations during the luteal phase of the free-living and first controlled diet: Carotenoids, Lipids, Hormone Study<sup>1</sup>

Carotenoid	Free-living cycle	First controlled diet
	<i>nmol/L</i>	
$\alpha$ -Carotene	33 ± 1	41 ± 1
$\beta$ -Carotene	379 ± 11	534 ± 11
$\beta$ -Cryptoxanthin	398 ± 7	362 ± 5
Lutein/zeaxanthin	370 ± 9	526 ± 7
Anhydrolutein	71 ± 2	102 ± 2
Lycopene	564 ± 22	986 ± 24
Phytofluene	142 ± 6	232 ± 7
Phytoene	26 ± 2	44 ± 2
Total carotenoids	2074 ± 43	2946 ± 45
Retinol	1539 ± 24	1504 ± 28

<sup>1</sup> Least-squared  $\bar{x} \pm SE$  based on ANOVA models;  $n = 12$ . Midluteal days were selected because they are the last days of the blood specimen schedule for each cycle and thereby reflect  $\geq 21$  d of the carotenoid-rich controlled diet.

TABLE 6

Specific and total plasma carotenoids, retinol, total-cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations by phase of the menstrual cycle during the second controlled diet cycle: Carotenoids, Lipids, Hormone Study<sup>1</sup>

Luteal	Plasma carotenoid and cholesterol	Phase of the menstrual cycle			
		Menses	Early follicular	Late follicular	Luteal
80 ± 275	$\alpha$ -Carotene (nmol/L)	115 ± 4	113 ± 2	115 ± 4	115 ± 4
48 ± 22	$\beta$ -Carotene (nmol/L)	526 ± 15	538 ± 9	575 ± 11 <sup>2</sup>	564 ± 9
81 ± 217	Lutein/zeaxanthin (nmol/L)	496 ± 12	537 ± 7 <sup>2</sup>	551 ± 9 <sup>2</sup>	553 ± 9 <sup>2</sup>
51 ± 16	Anhydrolutein (nmol/L)	100 ± 5	115 ± 4 <sup>2</sup>	116 ± 4 <sup>2</sup>	131 ± 4 <sup>2</sup>
89 ± 103	Lycopene (nmol/L)	1023 ± 34	1060 ± 19	1023 ± 22	1135 ± 22 <sup>2</sup>
48 ± 16	Phytofluene (nmol/L)	227 ± 15	245 ± 9	236 ± 9	275 ± 9 <sup>2</sup>
	Phytoene (nmol/L)	39 ± 4	50 ± 2 <sup>2</sup>	44 ± 2	48 ± 2 <sup>2</sup>
	Total carotenoids (nmol/L)	2936 ± 74	3097 ± 43	3106 ± 52	3275 ± 74 <sup>2</sup>
	Retinol (nmol/L)	1458 ± 38	1504 ± 21	1444 ± 28	1598 ± 25 <sup>2</sup>
	Total cholesterol (mmol/L)	4.34 ± 0.07	4.38 ± 0.03	4.36 ± 0.04	4.28 ± 0.04 <sup>2</sup>
	LDL cholesterol (mmol/L)	2.67 ± 0.05	2.70 ± 0.04	2.61 ± 0.04	2.58 ± 0.04 <sup>2</sup>
	HDL cholesterol (mmol/L)	1.41 ± 0.02	1.42 ± 0.01	1.50 ± 0.01 <sup>2</sup>	1.45 ± 0.02 <sup>2</sup>

<sup>1</sup> Least-squared  $\bar{x} \pm SE$  based on ANOVA models;  $n = 12$ .

<sup>2</sup> Significant percentage change since menses in the second controlled diet cycle,  $P < 0.05$ .

0.004) and by 21% ( $P = 0.006$ ), respectively, during the midluteal phase. Compared with the menses, plasma phytoene concentrations were higher during the early follicular (by 29%,  $P = 0.002$ ) and midluteal (by 24%,  $P = 0.02$ ) phases, respectively. Finally, total plasma carotenoid concentrations were higher by 12% at the midluteal phase than at menses ( $P = 0.0005$ ). (Note that plasma  $\beta$ -cryptoxanthin concentrations were not reported because cryptoxanthin intake was negligible during the CDI and CDII cycles and gradually dropped from 449 to 346 nmol/L at menses in the CDI to the CDII cycle, respectively, with a final concentration of 335 nmol/L at the midluteal phase of the CDII cycle.)

Plasma retinol concentrations were consistently higher at the midluteal phase than at menses in all three cycles (by 6% in the free-living, by 11% in the CDI, and by 10% in the CDII cycle,  $P \leq 0.004$ ). Plasma total- and LDL-cholesterol concentrations were higher during the early follicular than during the midluteal phase ( $P < 0.05$  for total, and  $P < 0.01$  for LDL cholesterol, respectively). Plasma HDL-cholesterol concentrations were higher during the late follicular than during the menses ( $P < 0.005$ ).

Additional models that adjusted for plasma total- or LDL-cholesterol concentrations did not alter the fluctuation of individual carotenoid and retinol concentrations (data not shown). The major effect of these adjustments partitioned variation that was unaccounted for, which reduced the error around the mean and increased the already significant  $P$  values.

## DISCUSSION

The purpose of this study was to examine the fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle in healthy, premenopausal women under controlled diet conditions. With this overall aim in mind, women with confirmed ovulation in the free-living cycle were placed on a standardized, carotenoid-rich diet in the CDI cycle to increase total carotenoid intake from an average of 6 mg/d to 10 mg/d. Total dietary carotenoid intake in the free-living cycle was comparable with total dietary carotenoid intake in a national sample of women of reproductive age (24). The 58% increase

in total carotenoid intake from the free-living to the CDI cycles was associated with a 42% increase in total plasma carotenoid concentrations by the midluteal phase of the CDI cycle.

In the CDII cycle, all plasma carotenoid concentrations were at their lowest at menses. Although plasma  $\alpha$ -carotene concentrations did not change from menses, plasma  $\beta$ -carotene concentrations peaked at the late follicular phase. Plasma concentrations of lutein/zeaxanthin and anhydrolutein were consistently higher after menses, with the peak for anhydrolutein occurring at the midluteal phase. Plasma lycopene, phytofluene, and phytoene peaked at the midluteal phase, similar to plasma retinol concentrations. Results from analysis of variance (ANOVA) models that adjusted for plasma total or LDL cholesterol increased the level of significance.

Plasma total- and LDL-cholesterol concentrations peaked at the early follicular phase and declined thereafter, whereas HDL cholesterol peaked at the late follicular phase. Because  $\beta$ -carotene and lycopene are predominantly carried on the LDL, these carotenoids peaked after the LDL peak. In contrast, lutein/zeaxanthin, which is carried more evenly on the HDL and LDL, was higher at the early follicular phase than at menses and peaked before its HDL carrier. Therefore, individual plasma carotenoids did not fluctuate with their lipoprotein carriers.

This is the first study to examine the fluctuation of plasma carotenoid and retinol concentrations by phase of the menstrual cycle under controlled diet conditions. In previous studies, plasma carotenoid concentrations did not vary with the menstrual cycle after adjustment for plasma cholesterol concentrations (18, 19). Both studies relied on subject-reported menses days to estimate blood sampling schedules. Selected hormones were measured in serum or in urine, but neither study had a comprehensive set of hormones (estradiol, luteinizing hormone, and progesterone) to determine ovulation nor as frequent blood draws with simultaneous measurement of the carotenoids, lipoproteins, and hormones as in this study. Prior research relied on food-diary record collection at select intervals during the cycle or cycles to estimate dietary carotenoid intake. Therefore, these methodologic differences could have influ-

enced the ability to detect variation in plasma carotenoid concentrations by phase of the menstrual cycle.

Whereas this study has certain methodologic strong points, there are several caveats. First, the sample size for this study was small, and therefore the statistical analyses relied heavily on assumptions of normality from the ANOVA modeling. We could not address these assumptions further without more participants. Second, the analysis focused on data from the CDII cycle; however, it would have been beneficial to have data from an additional cycle to reproduce the findings. Third, the cyclic increase in specific plasma carotenoid concentrations varied from 8% to 29%. Whether all the significant variation was biologically meaningful would be contingent on a better understanding of individual carotenoid metabolism and of the variation in carotenoid status over the life cycle of a woman. Note that the documented percentage change in plasma lipoprotein concentrations by phase of the menstrual cycle has not been as large as that for the carotenoids.

These findings may have implications for a cohort study of premenopausal women. Blood drawing schedules are not typically coordinated by phase of the cycle, but rather a calendar day sequence is established that does not correspond to the same phase of the cycle across the cohort. For example, women who are in the early follicular phase for the blood draw day have 8% higher plasma lutein/zeaxanthin concentrations than at menses, and likewise, those in the late follicular or luteal phase have 11% higher concentrations than at menses (Figure 1). Therefore, women would be potentially misclassified into categories of plasma lutein/zeaxanthin distribution, which would reduce the ability to estimate the putative plasma carotenoid-disease relation. Alternatively, blood collection could be synchronized to the first day of menses of each woman to reduce potential misclassification, or the date of her last menstrual period could be reported at the time of the blood draw to adjust plasma concentrations.

In summary, this is the first controlled diet study to show variation in plasma carotenoid concentrations with plasma lipoprotein and serum hormone concentrations by phase of the menstrual cycle. Individual plasma carotenoid concentrations

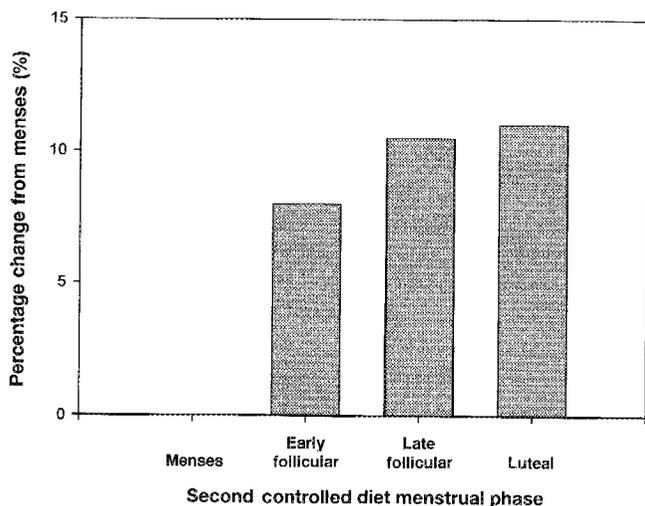


FIGURE 1. Percentage change in plasma lutein/zeaxanthin concentrations by phase of the menstrual cycle.  $n = 12$ .

were at their lowest at menses and peaked thereafter. Plasma retinol concentrations repeatedly peaked at the midluteal phase of all three cycles. Further investigation of the fluctuation of the plasma carotenoids is warranted in a larger sample population.

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## REFERENCES

1. Willett WC, Hunter DJ. Vitamin A and cancers of the breast, large bowel, and prostate: epidemiologic evidence. *Nutr Rev* 1994;52(suppl):S53-9.
2. Howe GR, Hirohata T, Hislop TG, et al. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. *J Natl Cancer Inst* 1990;82:561-9.
3. Krinsky NI. Effects of carotenoids in cellular and animal systems. *Am J Clin Nutr* 1991;53(suppl):238S-46S.
4. Moon RC. Vitamin A, retinoids and breast cancer. *Adv Exp Med Biol* 1994;364:101-7.
5. Sporn MB, Roberts AB. Role of retinoids in differentiation and carcinogenesis. *Cancer Res* 1983;43:3034-40.
6. Bendich A. Carotenoids and the immune response. *J Nutr* 1989;119:112-5.
7. Krinsky NI. Antioxidant functions of carotenoids. *Free Radic Biol Med* 1989;7:617-35.
8. Krinsky NI. Actions of carotenoids in biological systems. *Annu Rev Nutr* 1993;13:561-87.
9. Schultz TD, Chew BP, Seaman WR, Lueddecke LO. Inhibitory effect of conjugated dienoic derivatives of linoleic acid and beta-carotene of the in vitro growth of human cancer cells. *Cancer Lett* 1992;63:125-33.
10. Bertram JS. Inhibition of chemically induced neoplastic transformation by carotenoids: mechanistic studies. *Ann N Y Acad Sci* 1993;686:161-76.
11. Bertram JS, Pung A, Churley M, et al. Diverse carotenoids protect against chemically induced neoplastic transformation. *Carcinogenesis* 1991;12:671-8.
12. Simpson KL. Relative value of carotenoids as precursors of vitamin A. *Proc Nutr Soc* 1983;42:7-17.
13. Krinsky NL, Cornwell DG, Oncley JL. The transport of vitamin A and carotenoids in human plasma. *Arch Biochem Biophys* 1958;73:233-46.
14. Johnson EJ, Russell RM. Distribution of orally administered  $\beta$ -carotene among lipoproteins in healthy men. *Am J Clin Nutr* 1992;56:128-35.
15. Mattsson LA, Silfverstolpe G, Samsioe G. Lipid composition of serum lipoproteins in relation to gonadal hormones during the menstrual cycle. *Eur J Obstet Gynecol Reprod Biol* 1984;17:327-35.
16. Lyons-Walls PM, Choudhury N, Gerbrandt EA, Truswell AS. Increase in high density lipoprotein cholesterol at ovulation in healthy women. *Atherosclerosis* 1994;150:171-8.
17. Schejf CPT, Van Der Moren MJ, Doesburg WH, Thomas CMG, Rolland R. Differences in serum lipids, lipoproteins, sex hormone binding globulin and testosterone between the follicular and the luteal phase of the menstrual cycle. *Acta Endocrinol* 1993;129:130-3.
18. Tangney C, Brownie C, Wu SM. Impact of menstrual periodicity on serum lipid levels and estimates of dietary intakes. *J Am Coll Nutr* 1991;10:107-13.
19. Rock CL, Demitrack MA, Rosenwald EN, Brown MB. Carotenoids

- and menstrual cycle phase in young women. *Cancer Epidemiol Biomarkers Prev* 1995;4:283-8.
20. Metropolitan Life Insurance Company. 1983 height and weight tables. *Stat Bull Metrop Insur Co* 1984;64:2-9.
  21. Olson BR, Forman MR, Raymond E, et al. The relationship between menstrual cycle symptoms and sodium balance in normal women. *Ann Intern Med* (in press).
  22. Lanza E, Schatzkin A, Ballard-Barbash R, Clifford C, Paskett E, The Polyp Prevention Trial Study Group. The Polyp Prevention Trial II: dietary intervention program and participant baseline dietary characteristics. *Cancer Biomarkers Prev* (in press).
  23. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J Am Diet Assoc* 1993;93:284-96.
  24. University of Minnesota. Minnesota Nutrient Data System. Version 2.4. Minneapolis: University of Minnesota, 1992.
  25. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E. The development and application of a carotenoid database for fruits, vegetables, and selected multi-component foods. *J Am Diet Assoc* 1993;93:318-23.
  26. Munabi AK, King D, Bender S, Bustillo M, Dorfmann A, Schulman JD. Small increases in circulating luteinizing hormone concentrations shortly before human chorionic gonadotropin are associated with reduced in vitro fertilization pregnancy rate. *J In Vitro Fert Embryo Transf* 1990;7:310-3.
  27. Gidez LT, Miller GH, Burnstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res* 1982;23:1206-23.
  28. Muesing RA, Miller VT, LaRosa JC, Stoy DB, Phillips EA. Effects of unopposed conjugated equine estrogen on lipoprotein composition and apolipoprotein E distribution. *J Clin Endocrinol Metab* 1992;75:1250-4.
  29. NHLBI, NIH, DHHS Laboratory Methods Committee of the Lipid Research Clinics Program. In: Hainline A, Karon J, Lippel K, eds. *Manual of laboratory operations, lipid research clinics program. Vol 1. Lipid and lipoprotein analysis, revised 1982.* Washington, DC: US Government Printing Office.
  30. Friedewald WT, Levy RI, Fredrickson D. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-504.
  31. Khachik F, Beecher GR, Goli MB, Lusby WR Jr, Smith JC. Separation and identification of carotenoids and their oxidant products in the extracts of human plasma. *Anal Chem* 1992;64:2111-22.
  32. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. NHANES III: increasing prevalence of overweight among US adults. *JAMA* 1994;272:205-11.