

## $\alpha$ -Tocopherol Concentrations in Plasma but not in Lipoproteins Fluctuate during the Menstrual Cycle in Healthy Premenopausal Women<sup>1</sup>

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**ABSTRACT** Because premenopausal women experience cyclic fluctuations of plasma carotenoids and their lipoprotein carriers, it was hypothesized that plasma  $\alpha$ -tocopherol (A-T) fluctuates by phase of the menstrual cycle. Twelve free-living women, with a confirmed ovulatory cycle, were given a controlled diet for two consecutive menstrual cycles. Blood was drawn during the menses, early follicular, late follicular and luteal phases to simultaneously measure serum hormones, plasma lipoproteins and A-T concentrations, and A-T distribution in the lipoprotein fractions. Plasma A-T concentrations were significantly lower during menses than during the luteal phase by ~12% in each controlled diet cycle ( $P < 0.001$ ). Adjustment for serum cholesterol and triglyceride concentrations did not alter these findings. The distributions of A-T in lipoprotein cholesterol fractions were not significantly different by menstrual phase. From 61 to 62% of A-T was concentrated in the LDL fraction, with another 9–14% in HDL<sub>2</sub>, 17–22% in HDL<sub>3</sub> and the remaining 6–8% in VLDL+ IDL. There were no significant differences in lipoprotein cholesterol fractions by menstrual phase, except for a significant increase ( $P = 0.03$ ) in HDL<sub>2</sub> cholesterol from the early follicular to the late follicular phase. Spearman rank correlations from data during the second controlled diet month showed A-T in HDL<sub>2</sub> in the late follicular phase was positively correlated with HDL cholesterol in the early follicular ( $r = 0.88$ ), late follicular ( $r = 0.86$ ) and luteal phases ( $r = 0.86$ ) and with luteal apolipoprotein (ApoA-1) level ( $r = 0.90$ ), and luteal HDL<sub>2</sub> cholesterol ( $r = 0.83$ ). A-T in HDL<sub>3</sub> in the early follicular phase was negatively correlated with HDL<sub>2</sub> cholesterol ( $r = -0.96$ ) and ApoA-1 ( $r = -0.85$ ), whereas luteal A-T in HDL<sub>3</sub> was correlated with luteal HDL<sub>3</sub> cholesterol ( $r = -0.79$ ). Late follicular A-T in VLDL was positively correlated with early follicular HDL<sub>3</sub> cholesterol and late follicular HDL<sub>3</sub> cholesterol ( $r = 0.83$ ). Fluctuations of A-T concentrations by phase of the menstrual cycle should be taken into consideration in future research concerning premenopausal women and the risk of chronic disease. *J. Nutr.* 128: 1150–1155, 1998.

**KEY WORDS:** • *tocopherol* • *vitamin E* • *premenopausal* • *humans* • *lipoproteins*

In the past few years, there has been a resurgence of interest in the role of antioxidants and in coronary artery disease. From an interim analysis of 16 of 33 populations participating in the WHO study to monitor trends and determinants in cardiovascular disease, there was a strong inverse correlation between lipid standardized vitamin E concentrations and risk of coronary artery disease mortality across the study communities (Gey et al. 1991). It has been postulated that vitamin E protects against coronary artery disease through its ability to inhibit the formation of oxidized LDL, which possesses pro-ath-

erogenic properties (Steinberg et al. 1989). Furthermore, the vitamin E concentration in the LDL was inversely associated with the development of stenoses in coronary arteries; thus a reduced ratio of vitamin E/LDL may contribute to clinically manifest coronary artery disease (Regnstrom et al. 1995). Results from other observational epidemiologic studies on cardiovascular disease risk and plasma vitamin E levels are not totally consistent (Manson et al. 1993). The relationship of plasma vitamin E and specific cancers is even more controversial (Flagg et al. 1995). These inconsistent findings could be due to normal variation in plasma levels of vitamin E as well as the range in concentration of vitamin E in the various lipoproteins. Understanding the determinants of distribution of vitamin E in the lipoproteins and how this distribution relates to total plasma levels of vitamin E may lead to a better understanding of how vitamin E protects LDL from oxidation and the potential role of vitamin E in cell proliferation and signal transduction (Traber and Packer 1995).

Although vitamin E occurs in nature in eight different

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<sup>3</sup> Abbreviations used: ApoA-1, apolipoprotein A-1; A-T,  $\alpha$ -tocopherol; CD1, first month of controlled diet feeding; CD2, second month of controlled diet feeding; FL, free-living (no controlled diet); HDL cholesterol, HDL-C; LDL cholesterol, LDL-C; LH, luteinizing hormone; TE, tocopherol equivalents.

forms,  $\alpha$ -tocopherol (A-T)<sup>3</sup> is the form found in highest concentrations in human plasma and tissue and has the highest biological activity (Kayden and Traber 1993). A-T is transported in lipoproteins (McCormick et al. 1960). Recently, it has been demonstrated that plasma lipoprotein levels fluctuate by phase of the menstrual cycle (Cohn et al. 1992). This cyclic fluctuation in lipoproteins could be associated with fluctuation in plasma antioxidant concentrations. As part of a controlled feeding study of the fluctuation of plasma lipoproteins and serum hormones by phase of the menstrual cycle, plasma carotenoids and A-T were examined. Hormones, lipoproteins, A-T and carotenoids were measured simultaneously for 2–3 consecutive days of each phase of the menstrual cycle. The fluctuations in plasma lipoprotein and carotenoid concentrations were recently published (Forman et al. 1996, Muesing et al. 1996). LDL cholesterol (LDL-C) levels were lowest during the luteal phase, HDL-C levels were highest at the late follicular phase, and ApoA-I levels were highest during the luteal phase (Muesing et al. 1996). Carotenoids were lowest during menses, with concentrations of lutein/zeaxanthin and its metabolite, anhydrolutein, higher at all three phases (early follicular, late follicular and luteal) than during menses (Forman et al. 1996). Plasma  $\beta$ -carotene peaked at the late follicular phase, whereas plasma lycopene, phytoene, phytofluene and retinol concentrations peaked at the luteal phase (Forman et al. 1996). The objective of this paper is to report the cyclic fluctuations of  $\alpha$ -tocopherol, the major circulating form of vitamin E, and the distribution of  $\alpha$ -tocopherol in lipoproteins by phase of the menstrual cycle during a controlled dietary study.

## SUBJECTS AND METHODS

Female volunteers were recruited from the Beltsville, MD area. After an initial screening by phone, potential participants visited the National Institutes of Health (NIH) Clinical Center for a physical and gynecologic exam, blood biochemistries and an in-person interview (Forman et al. 1996). Participants met the following eligibility criteria: 1) aged 20–34 y; 2) non-smokers; 3) not pregnant, not receiving hormone preparations or breast feeding in the past 6 mo; 4) no history or clinical signs of gynecologic problems; 5) within 20% of weight for height based on age and gender-specific reference values; 6) plasma cholesterol, triglyceride and hemoglobin concentrations within the normal range for women of reproductive age; 7) not consuming a restricted diet or a regular user of vitamin-mineral supplements; and 8) willingness to stop supplementation and to abstain from alcohol use during the controlled diet study. The protocol was approved by the internal review boards of the George Washington University School of Medicine, the National Cancer Institute and the Beltsville Human Nutrition Research Center (BHNRC), USDA.

Of the 14 women who began the controlled diet study, two were removed from data analysis because one experienced an anovulatory cycle, and another was a plasma nonresponder to dietary carotenoids (Forman et al. 1996). Three others were removed from the analysis of the distribution of A-T in lipoproteins because their lipoprotein fractions were stored inappropriately (4°C). Thus data were available to describe plasma fluctuations of A-T in 12 women and the A-T distribution in lipoproteins in 9 of these 12 women. There were no significant differences in major characteristics of these two groups of women (Table 1).

**Study design.** The study was designed as a free-living (FL) study of one menstrual cycle to confirm ovulation followed by a controlled diet study for two consecutive menstrual cycles (Forman et al. 1996). The diet study was conducted at the BHNRC, USDA. Each woman began the free-living study on her first menses day. Food and beverage records were kept during this menstrual cycle. The controlled diet study consisted of a 7-d repeat menu cycle. The daily diet was composed of 36% of energy from fat (polyunsaturated:saturated ratio of 0.53), 19% from protein, 55% from carbohydrates and 3.4 g of fiber/100 kJ. The dietary intake of vitamin E during the two controlled diet periods was  $8.1 \pm 1.1$  mg tocopherol equivalents (TE). Dietary

TABLE 1

Characteristics of the study participants<sup>1</sup>

	Serum A-T <sup>2</sup> Analysis	Distribution of A-T Analysis
<i>n</i>	12	9
Age, y	27 $\pm$ 3	28 $\pm$ 3
Height, cm	166 $\pm$ 5	167 $\pm$ 5
Weight, kg	58 $\pm$ 6	58 $\pm$ 7
Body mass index, kg/m <sup>2</sup>	21 $\pm$ 2	21 $\pm$ 2
Education, y	13 $\pm$ 2	12 $\pm$ 0.7
Cycle length, d	27 $\pm$ 2	26 $\pm$ 1

<sup>1</sup> Values are means  $\pm$  SD.

<sup>2</sup> A-T,  $\alpha$ -tocopherol.

intake of vitamin E in the FL month was  $8.2 \pm 5.2$  mg for all twelve women and  $8.8 \pm 4.9$  mg TE for the nine women.

**Measurement of serum hormones, plasma lipids, and  $\alpha$ -tocopherol on the lipoprotein fractions.** Blood samples were collected between 0600 and 0700 h from women who had fasted for >12 h. Samples were collected in EDTA and non-EDTA glass vacutainers for plasma A-T/lipoprotein and serum hormone analyses, respectively. Blood drawing for plasma concentrations of lipoproteins and A-T occurred on the following days of the menstrual cycle: 1) menses, menses d 1 and 2; 2) early follicular, menses d 4–6; 3) late follicular, menses d 11 through 1 d after serum luteinizing hormone (LH) concentration was >30 mIU/L, and 4) midluteal, menses d 7–8 post-LH surge. An ovulatory cycle was defined as a serum LH concentration  $\geq 30$  U/L at the time of LH surge and a serum progesterone concentration  $\geq 13$  nmol/L at the midluteal phase.

Blood for the lipoprotein fractions was collected during the second menstrual cycle of the controlled feeding study on the following days: 1) menses d 4 of the early follicular, 2) the day of ( $n = 7$ ) or day after ( $n = 2$ ) the LH surge for the late follicular, and 3) either d 7 or 8 after the LH surge for the luteal phase.

Hormone determinations were performed by the Immunoassay Laboratory of the Genetics and In Vitro Fertilization Institute, Fairfax, VA on the same day as the blood draw. Serum progesterone concentrations were measured by direct RIA (Coat-A-Count Assay, Diagnostic Products, La Jolla, CA). Serum LH was quantified by monoclonal Immuno-Radio-Metric-Assay Sero (Randolph, MA) as described by Munabi et al. (1990).

HDL and HDL<sub>3</sub> fractions were isolated at the GWU Lipid Research Clinic Laboratory on the day of the blood draw by using the precipitation procedures of Gidez et al. (1982) and Muesing et al. (1992). Very low plus intermediate density lipoproteins (VLDL + IDL) and LDL + HDL fractions were isolated by preparative ultracentrifugation (100,000  $\times$  g for 18 min at 10°C) at a density of 1.019 kg/L, thus avoiding the confounding of the IDL contribution to the LDL and HDL fractions. Cholesterol and triglycerides were determined enzymatically (Hainline et al. 1974). LDL concentration was the difference between (HDL + LDL) minus HDL; HDL<sub>2</sub> concentration was the difference between the HDL and HDL<sub>3</sub> concentrations. The plasma and isolated fractions were stored frozen at  $-80^\circ\text{C}$ , and then all samples from an individual were analyzed sequentially as a set. The laboratory maintains standardization with the Centers for Disease Control and Prevention for analysis of cholesterol, triglycerides and HDL-C.

Plasma A-T analyses were performed by the NCL, USDA BHNRC (Beltsville, MD), employing an HPLC system with a C18 reversed-phase column and a gradient mobile phase that varied in acetonitrile, dichloromethane and hexane (Khachick 1992). The limit of detection for A-T was 7.2 pmol/L. Analysis of A-T in the lipoprotein fractions was performed by the Jean Mayer USDA Human Research Nutrition Center on Aging at Tufts University as described by Forman et al. (1998) and Ribaya-Mercando et al. (1995).

**Statistical analysis.** In the initial analysis, measures of central tendency for plasma concentrations of A-T, total cholesterol, LDL-C and HDL-C were calculated for each phase (menses, early follicular, late follicular and luteal) of the menstrual cycle and each of the

following diet cycles: FL, free-living menstrual cycle; CD1, first controlled diet menstrual cycle; and CD2, second controlled diet menstrual cycle. Plasma A-T concentrations of each subject were averaged over 2–3 consecutive days during the menses, early follicular and luteal phases, respectively. In the late follicular phase, measurements on the two menses days before plus the day of the LH surge were averaged to estimate plasma A-T concentrations. Daily plasma lipoprotein concentrations were matched with plasma A-T concentrations by phase of the menstrual cycle.

After the initial analysis, regression modeling with categorical variables as predictors was used to compare mean individual concentrations by phase of the menstrual cycle (SAS, PROC GLM, SAS Institute, Cary, NC). The regression model included a separate intercept (fixed effect) 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 is 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 ( $\pm$  SEM) for A-T by phase of the menstrual cycle were generated from the models. Adjustment of plasma tocopherol concentrations for total cholesterol, LDL-C, HDL-C and triglycerides was accomplished by dividing each woman's mean tocopherol concentration by her mean lipoprotein concentration at each phase of the menstrual cycle.

Similar regression modeling of the cyclic fluctuation of plasma A-T concentration was used to estimate and compare A-T in each lipoprotein fraction [VLDL + IDL; LDL; HDL<sub>3</sub>; HDL<sub>2</sub> (i.e., HDL – HDL<sub>3</sub>)] by phase of the last cycle. An adjustment for VLDL + IDL and LDL A-T concentrations was added to the model to correct for the differences in the sum of the lipoprotein A-T concentrations in contrast with the actual plasma A-T concentrations. The sum of the lipoprotein concentrations ranged from 97 to 101% of the plasma concentrations. The percentage of A-T in each lipoprotein was calculated by summing the least square mean values for A-T in VLDL + IDL, LDL, HDL<sub>2</sub> and HDL<sub>3</sub>, then dividing A-T in a specific lipoprotein fraction by the sum and multiplying by 100. All differences were considered significant when the two-sided *P*-value was  $< 0.05$ .

## RESULTS

There were no significant differences in plasma A-T by menstrual cycle phase during the free-living time (Table 2). In CD1, A-T was lower at menses than during the early follicular ( $P = 0.0001$ ), late follicular ( $P = 0.0001$ ) or luteal ( $P = 0.0002$ ) phases. In CD2, A-T was higher in the luteal phase than in the early follicular phase ( $P = 0.004$ ), late follicular phase ( $P = 0.008$ ) or during menses ( $P = 0.0001$ ). During both controlled diet months, plasma A-T during menses was  $\sim 12\%$  lower than during the luteal phase ( $P = 0.002$ , CD1 and  $P = 0.0001$ , CD2). Adjustment of plasma A-T for plasma total cholesterol and triglyceride concentrations did not alter the results (Table 2).

There was no significant difference in the concentration of A-T in each lipoprotein cholesterol fraction by phase of the menstrual cycle (Table 3). The majority of A-T was found in the LDL fraction (12.00–12.91  $\mu\text{mol/L}$ ), whereas the HDL fraction contained approximately half as much (6.16–6.63  $\mu\text{mol/L}$ ). Between 61 and 62% of the A-T was in the LDL fraction, with another 6–8% in the VLDL + IDL fraction and 31–32% in the HDL fraction. A-T in the HDL<sub>2</sub> fraction varied from 9 to 14% and in the HDL<sub>3</sub> fraction from 17 to 22% over the cycle. Among the nine women studied, the only significant difference in lipoprotein cholesterol fractions during these three menstrual phases, early follicular, late follicular and luteal, was the higher proportion in the HDL<sub>2</sub> fraction in the late follicular than in the early follicular phase ( $P = 0.03$ ).

Table 4 shows the Spearman rank correlation coefficients between A-T in the lipoprotein fractions and plasma lipoprotein levels. A-T in HDL<sub>2</sub> in the late follicular phase was correlated with HDL-C in the early follicular, late follicular and

luteal phases, ApoA-1 in the luteal phase and HDL<sub>2</sub>-C in the luteal phase. A-T in HDL<sub>3</sub> in the early follicular phase was inversely related to ApoA-1 in the early follicular and luteal phases, and HDL<sub>2</sub>-C in all three phases, whereas A-T in HDL<sub>3</sub> in the luteal phase was correlated with HDL<sub>3</sub>-C only during the luteal phase. Finally, A-T in VLDL in the late follicular phase was correlated with HDL<sub>3</sub>-C levels in the early follicular and luteal phases.

## DISCUSSION

The purpose of this investigation was to determine whether A-T levels fluctuate by phase of the menstrual cycle in healthy premenopausal women. Because diet can alter both plasma A-T and lipoprotein levels and thus obscure any true differences, this study was conducted under controlled dietary conditions. In both controlled diet cycles, CD1 and CD2, plasma A-T concentrations were 12% lower during menses than during the luteal phase ( $P = 0.0002$ ,  $P = 0.0001$ ). Thus this is the first study to report on the menstrual periodicity of circulating A-T under controlled dietary conditions.

In a study of nine amenorrheic women, Tagney et al. (1991) found no fluctuations in plasma A-T under free-living conditions. Our results in the FL month are in agreement with these findings. Although dietary vitamin E intake was similar ( $\sim 8.1$  mg TE) during all three menstrual cycles (FL, CD1 and CD2), the higher level of variation in the FL month [SD = 5.2 mg TE (FL) vs. 1.1 mg TE (CD2)] could have reduced the chances of seeing any differences. The lower amount of circulating A-T during menses ( $\sim 12\%$ ) compared with the luteal phase was found in both CD cycles. The slight increase in A-T in the luteal phase of the second month of controlled feeding could be due to the sustained increase in carotenoid-rich foods. Several studies have shown that an increase in carotenoid intake leads to an increase in serum tocopherol level (Fontham et al. 1995, Goodman et al. 1994). The higher level of circulating A-T in the luteal phase compared with during menses mimics the recently reported findings in plasma carotenoid and suggests that levels of available circulating micronutrients might be reduced during menses along with the lower immune status during this time (Forman et al. 1996). Because cyclic fluctuations in lipoproteins could be responsible for the observed differences in A-T, we also adjusted all data for plasma total cholesterol and triglyceride levels. These adjustments did not alter the findings.

The decrease in plasma A-T during menses could be due to either a decrease in the incorporation of A-T into VLDL during its synthesis, resulting in lower blood levels and an increase in the liver, or to an increase in uptake of A-T by peripheral tissue. The former hypothesis seems more probable because LDL, the major carrier of A-T, is highest during the menses/early follicular phases.

A-T in the plasma is in a constant state of flux. Circulating lipoproteins containing A-T are taken up by the liver and incorporated into newly synthesized VLDL and then returning to the plasma with  $\sim 74$   $\mu\text{mol/d}$  of A-T reincorporated into the plasma (Traber 1997). Thus, the body expends considerable effort to maintain concentrations of A-T in the plasma for delivery to tissues (Cohn et al. 1992). Little is known about the regulation and transfer of A-T between plasma and tissue. Recent studies in rats revealed that ovarian levels of A-T increased during the luteal phase after steriogenesis. Therefore, the antioxidant reserve of the ovary may be in a dynamic state that is under endocrine regulation (Aten et al. 1994). Our finding of the highest concentration of plasma A-T during the luteal phase might be similar to the findings of Aten et al. and

**TABLE 2**

*Concentrations of plasma  $\alpha$ -tocopherol in women by phase of the menstrual cycle during free-living (FL) and controlled dietary (CD) conditions*

Study period	Phase of the menstrual cycle <sup>1</sup>			
	Menses	Early follicular	Late follicular	Luteal
$\alpha$ -Tocopherol, $\mu\text{mol/L}$				
FL <sup>2</sup>	18.6 $\pm$ 0.6	18.7 $\pm$ 0.6	17.3 $\pm$ 0.4)	17.4 $\pm$ 0.3
FL <sup>3</sup>	18.4 $\pm$ 0.7	18.7 $\pm$ 0.7	17.5 $\pm$ 0.	17.8 $\pm$ 0.5
FL <sup>4</sup>	18.4 $\pm$ 0.7	18.6 $\pm$ 0.7	17.3 $\pm$ 0.	17.6 $\pm$ 0.5
CD1 <sup>2</sup>	17.0 $\pm$ 0.4	19.7 $\pm$ 0.25 <sup>†</sup>	19.6 $\pm$ 0.35 <sup>†</sup>	19.1 $\pm$ 0.35 <sup>†</sup>
CD1 <sup>3</sup>	17.6 $\pm$ 0.4	19.6 $\pm$ 0.35*	19.8 $\pm$ 0.35 <sup>†</sup>	19.1 $\pm$ 0.35 <sup>†</sup>
CD1 <sup>4</sup>	17.1 $\pm$ 0.4	19.4 $\pm$ 0.35*	19.6 $\pm$ 0.35*	19.3 $\pm$ 0.35*
CD2 <sup>2</sup>	18.6 $\pm$ 0.4	19.6 $\pm$ 0.36*	19.2 $\pm$ 0.36 <sup>†</sup>	20.8 $\pm$ 0.46 <sup>†</sup>
CD2 <sup>3</sup>	18.6 $\pm$ 0.4	19.6 $\pm$ 0.26*	19.3 $\pm$ 0.46 <sup>†</sup>	21.0 $\pm$ 0.35 <sup>†</sup>
CD2 <sup>4</sup>	18.6 $\pm$ 0.4	19.6 $\pm$ 0.26*	19.2 $\pm$ 0.46 <sup>†</sup>	20.8 $\pm$ 0.36 <sup>†</sup>
Cholesterol, $\text{mmol/L}$				
FL	4.27 $\pm$ 0.62	4.31 $\pm$ 0.625*	4.14 $\pm$ 0.625*	4.09 $\pm$ 0.527*
CD1	4.21 $\pm$ 0.07	4.51 $\pm$ 0.045*	4.39 $\pm$ 0.055*	4.28 $\pm$ 0.057*
CD2	4.34 $\pm$ 0.07	4.38 $\pm$ 0.03	4.36 $\pm$ 0.04	4.28 $\pm$ 0.04
Triglycerides, $\text{mmol/L}$				
FL	0.59 $\pm$ 0.19	0.60 $\pm$ 0.19	0.56 $\pm$ 0.17	0.56 $\pm$ 0.23
CD1	0.58 $\pm$ 0.04	0.63 $\pm$ 0.02	0.54 $\pm$ 0.03	0.56 $\pm$ 0.02
CD2	0.59 $\pm$ 0.04	0.58 $\pm$ 0.02	0.54 $\pm$ 0.02	0.56 $\pm$ 0.02

<sup>1</sup> Values are least square means  $\pm$  SEM generated from the regression model,  $n = 12$ . FL, menstrual cycle during the free living month; CD1, menstrual cycle during the first month of controlled diet feeding; CD2, menstrual cycle during the second month of controlled diet feeding.

<sup>2</sup> A-T values not adjusted for plasma lipoproteins concentration.

<sup>3</sup> A-T values adjusted for total plasma cholesterol concentration.

<sup>4</sup> A-T values adjusted for plasma triglycerides concentration.

<sup>5</sup> Significantly different from menses.

<sup>6</sup> Significantly different from luteal.

<sup>7</sup> Significantly different from early follicular.

\*  $P \leq 0.001$ , <sup>†</sup>  $P \leq 0.0001$ .

reflects A-T transfer from either the ovary or other hormone-dependent tissue; however, this interpretation requires further investigation. Of course, there are physical collisions between lipoprotein particles, which could lead to some exchange of A-T between lipoproteins in plasma.

Because A-T levels fluctuate by phase of the menstrual

cycle, it is important that specimens for A-T measurements be collected at the same phase of the menstrual cycle or, at a minimum, data obtained during the last menstrual cycle should be used to adjust plasma concentrations. Failure to account for this component of variability (12%) could limit the utility of the data. Individuals would be potentially misclassified by

**TABLE 3**

*Concentration and distribution of  $\alpha$ -tocopherol in premenopausal women's plasma lipoprotein fractions during the early follicular, late follicular and luteal phases of the menstrual cycle in the second month of a controlled diet*

Lipoprotein fraction	Phase of the menstrual cycle <sup>1</sup>					
	Early follicular		Late follicular		Luteal	
	$\mu\text{mol/L}$	% <sup>2</sup>	$\mu\text{mol/L}$	% <sup>2</sup>	$\mu\text{mol/L}$	% <sup>2</sup>
VLDL + IDL	1.19 $\pm$ 0.38	6.0	1.75 $\pm$ 0.37	8.3	1.53 $\pm$ 0.28	7.8
LDL-C <sup>3</sup>	12.28 $\pm$ 1.10	61.9	12.91 $\pm$ 1.10	60.9	12.00 $\pm$ 0.89	60.9
HDL <sub>2</sub> -C	1.73 $\pm$ 0.72	8.7	3.74 $\pm$ 0.85	13.9	2.74 $\pm$ 0.69	13.9
HDL <sub>3</sub> -C	4.63 $\pm$ 0.72	22.3	2.79 $\pm$ 0.85	17.4	3.42 $\pm$ 0.69	17.4

<sup>1</sup> Least square means  $\pm$  SEM generated from regression model,  $n = 9$ .

<sup>2</sup> Percentage of  $\alpha$ -tocopherol in the lipoprotein cholesterol fractions.

<sup>3</sup> C, cholesterol.

TABLE 4

Spearman rank correlation coefficients between  $\alpha$ -tocopherol (A-T) concentration in the lipoprotein fractions and lipoprotein cholesterol (C) concentrations in the early follicular, late follicular and luteal phases of the menstrual cycle during the second month of a controlled diet<sup>1</sup>

A-T in lipoprotein fraction Lipoprotein Cholesterol	Menstrual phase	A-T in HDL <sub>2</sub> Late follicular	A-T in HDL <sub>3</sub> Early follicular	A-T in HDL <sub>3</sub> Luteal	A-T in VLDL Late follicular
HDL-C	Early follicular	0.88†			
HDL-C	Late follicular	0.86*			
HDL-C	Luteal	0.86*			
ApoA-1 <sup>2</sup>	Early follicular		-0.85†		
ApoA-1	Late follicular				
ApoA-1	Luteal	0.90†	-0.80*		
HDL <sub>2</sub>	Early follicular		-0.96*		
HDL <sub>2</sub>	Late follicular		-0.83*		
HDL <sub>2</sub>	Luteal	0.83*	-0.88†		
HDL <sub>3</sub>	Early follicular				0.83*
HDL <sub>3</sub>	Late follicular				
HDL <sub>3</sub>	Luteal			0.79*	0.83*

<sup>1</sup> Spearman rank correlation coefficient (*P*-values).

<sup>2</sup> ApoA-1, apolipoprotein A-1.

\* *P* < 0.01, † *P* < 0.01.

quartile of plasma A-T levels, thereby underestimating the risk of disease.

This is the first report of the distribution of A-T in HDL<sub>2</sub> and HDL<sub>3</sub> lipoprotein fractions. There were no significant differences in the distribution of A-T among the lipoprotein fractions during the menstrual cycle. The level of A-T in HDL<sub>2</sub> was highly correlated with HDL<sub>2</sub>-C and ApoA-1 levels, whereas the A-T level in HDL<sub>3</sub> was inversely correlated with HDL<sub>2</sub>-C and ApoA-1. A-T in the LDL fraction was not correlated with LDL-C levels. Mechanisms that regulate the partitioning of A-T among plasma lipoproteins classes are not well understood. The majority of A-T is carried in LDL and HDL in humans. Behrens et al. (1982) reported a significant difference in distribution between men and women. Men carried more A-T in LDL than HDL, whereas the opposite was true for women. It has been suggested that the difference might be explained by differences in lipoprotein levels between men and women. Since that publication in 1982, there have been six studies reporting distribution of A-T among lipoprotein fractions. Data for women alone were reported in four of these (Carelain et al 1992, Clevidence et al 1989, Kostner et al 1996, Ogihara et al 1988, Ribaya-Mercada et al. 1995, and Romanchik et al. 1995). In two studies, 50–53% of A-T was concentrated in the HDL fractions and 41–47% was found in the LDL fraction (Carelain et al. 1992, Ogihara et al. 1988). In the remaining two studies, a higher percentage of A-T was found in the LDL than the HDL fraction, similar to the percentages in males (Clevidence et al. 1989, Ribaya-Mercada et al 1995). These differences may be due to factors such as methodological procedures and/or differences in recoveries of lipoprotein fractions. Moreover, recent studies have shown a relatively higher proportion of A-T in the VLDL fraction, ranging from 21 to 25% compared with the 6–8% found in this study (Kostner et al. 1996, Ribaya-Mercada et al. 1995, Romanchik et al. 1995). The higher percentage could be due to the higher amount of VLDL-C present in plasma in postmenopausal women and men in previous studies in contrast with the lower levels of triglycerides and hence VLDL-C in the premenopausal women of this study.

The significant increase in HDL<sub>2</sub>-C in the late follicular phase is an important finding of this study. There has been

only one other investigation of lipoprotein subclasses during the menstrual cycle. Williams et al. (1994) found that HDL<sub>2a</sub>-C levels increase during the luteal phase. In that study, women did not consume a controlled diet and the menstrual cycle was determined by calendar days rather than serum hormone measurement. This latter factor could contribute to some imprecision in the estimate of phase-specific HDL-C levels. The findings in our study and that of Williams et al. are consistent with the observations that postmenopausal estrogen replacement therapy increases HDL<sub>2</sub>-C and that coronary heart disease risk may be increased when HDL<sub>2</sub> is decreased relative to HDL<sub>3</sub>-C (Johansson et al. 1991, Williams et al. 1993).

There were positive correlations between A-T in HDL<sub>2</sub> and HDL-C, HDL<sub>2</sub>-C and ApoA-1 levels, whereas A-T in HDL<sub>3</sub> was inversely related to ApoA-1, HDL<sub>2</sub>-C and positively correlated with HDL<sub>3</sub>-C levels. A-T in VLDL (late follicular) was positively correlated with HDL<sub>3</sub>-C (early follicular and luteal). There were no association between A-T in LDL and any lipoprotein cholesterol or protein. Two earlier studies in premenopausal women yielded similar findings (Behrens 1982, Clevidence and Lehmann 1989). A-T in HDL correlated highly with the amount of protein in HDL, but A-T in LDL was not associated with LDL protein (Behrens 1982). In the other study, A-T in HDL was positively correlated with ApoA and HDL-C (Clevidence and Lehmann 1989). Total serum A-T was also correlated with serum ApoA-1 and HDL-C, but not serum ApoB and LDL + VLDL-C. In contrast, a recent study in postmenopausal women found that A-T in HDL was not correlated with HDL-C, but A-T in LDL was positively correlated with LDL-C, and A-T in VLDL was positively correlated with VLDL-C (Ribaya-Mercada 1995). Traber et al. (1992) described a positive correlation between the HDL protein, ApoA-1, and the amount of A-T in HDL and suggested that A-T associates with the protein moiety of HDL. Ziouzenkova et al. (1996) found A-T to be positively correlated with total plasma cholesterol, but not A-T in LDL. Clearly, more research is required on the physiologic importance of this observation to understand what factors control the distribution of A-T in the lipoproteins.

In summary, A-T fluctuations during the menstrual cycle are similar to the reported fluctuations of plasma carotenoids,

which are lowest during menses and peak at the luteal phase (Forman et al. 1996). Furthermore, the magnitude of the changes for these antioxidants is larger than the cyclic fluctuation of lipoprotein cholesterol. The distribution of A-T in the lipoprotein cholesterol fractions [VLDL (6–8%), LDL (61–62%), HDL<sub>2</sub> (9–14%) and HDL<sub>3</sub> (17–22%)] is quite similar to the pattern for lutein and differs from the distribution for the hydrocarbon carotenoids, which are more concentrated in LDL (Forman et al. 1997). As with studies of lipids and carotenoids, studies of A-T in premenopausal women require consideration of menstrual periodicity. Our findings suggest the need for more studies in women to investigate the distribution of A-T in the HDL subclasses, especially because these subclasses appear to be influenced by hormone levels.

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