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**Abstract:** The purpose of this study was to assess the correlates and possible determinants of plasma vitamin A among middle-aged women in rural China. The vitamin A adequacy of the different diets at the five widely varying survey sites was also assessed. Patterns of correlations among dietary and biochemical measurements were strikingly different from previous studies in Western subjects. Plasma B-carotene was uncorrelated with B-carotene intake even after adjusting for potential confounders. In counties with low preformed vitamin A intake, plasma retinol correlated with plasma copper ( $P = 0.007$ ), which in turn was correlated with dietary intake of copper ( $P = 0.007$ ). In these counties plasma retinol was correlated with plasma B-carotene ( $P = 0.001$ ) and was increased 10% in women in the first 2 y of menopause ( $P = 0.028$ ). Plasma retinol and C-reactive protein levels are inversely correlated ( $r = -0.15$ ,  $P < 0.001$ ), indicating that vitamin A status and inflammatory response may be related but the causal direction is unknown. Despite low intake of vitamin A (county averages of 13–78% of recommended daily allowance [RDA]), and particularly of preformed vitamin A, only 3% of these women had plasma retinol levels below  $0.7 \mu\text{mol/L}$ , considered to indicate possible marginal deficiency. Plasma levels of retinol and B-carotene were more influenced by intrinsic factors such as menopause, lipid status, retinol requirements, and possibly copper status and inflammation than by extrinsic factors such as diet and lifestyle

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# Determinants of Plasma Retinol Concentrations of Middle-Aged Women in Rural China

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## ABSTRACT

The purpose of this study was to assess the correlates and possible determinants of plasma vitamin A among middle-aged women in rural China. The vitamin A adequacy of the different diets at the five widely varying survey sites was also assessed. Patterns of correlations among dietary and biochemical measurements were strikingly different from previous studies in Western subjects. Plasma *B*-carotene was uncorrelated with *B*-carotene intake even after adjusting for potential confounders. In counties with low preformed vitamin A intake, plasma retinol correlated with plasma copper ( $P = 0.007$ ), which in turn was correlated with dietary intake of copper ( $P = 0.007$ ). In these counties plasma retinol was correlated with plasma *B*-carotene ( $P = 0.001$ ) and was increased 10% in women in the first 2 y of menopause ( $P = 0.028$ ). Plasma retinol and C-reactive protein levels are inversely correlated ( $r = -0.15$ ,  $P < 0.001$ ), indicating that vitamin A status and inflammatory response may be related but the causal direction is unknown. Despite low intake of vitamin A (county averages of 13–78% of recommended daily allowance [RDA]), and particularly of preformed vitamin A, only 3% of these women had plasma retinol levels below  $0.7 \mu\text{mol/L}$ , considered to indicate possible marginal deficiency. Plasma levels of retinol and *B*-carotene were more influenced by intrinsic factors such as menopause, lipid status, retinol requirements, and possibly copper status and inflammation than by extrinsic factors such as diet and lifestyle.

Key words: *B*-carotene, plasma, retinol, copper, C-reactive protein, women, menopause

TABLE I.

LOCATION, DIETARY PATTERNS, AND COUNTY MEANS OF SELECTED NUTRIENT INTAKES OF WOMEN IN FIVE RURAL COUNTIES

County	CD	LC	SB	WA	YA
Province	Shanxi	Fujian	Sichuan	Xinjiang	Neimongol
Location	Plains	Coastal	Mountains	Plateau	High plateau
Diets	Noodles, numerous vegetables, millet, spices	Rice, numerous vegetables, fish and seafood, pork and eggs, spices	Rice, sweet potatoes, corn flour, green and pickled vegetables, hot spices	Wheat bread, milk-tea, mutton and beef, potatoes and carrots	Milk-tea, dairy products, wheat flour, mutton and beef, millet
Nutrient intakes (per day)*					
Energy (MJ)	8.1 ± 1.7 <sup>b</sup>	7.5 ± 1.6 <sup>ab</sup>	10.8 ± 3.1 <sup>c</sup>	6.7 ± 1.9 <sup>a</sup>	6.9 ± 1.7 <sup>a</sup>
Meat fat (g)	3 ± 6 <sup>a</sup>	9 ± 10 <sup>b</sup>	3 ± 7 <sup>a</sup>	11 ± 11 <sup>b</sup>	12 ± 9 <sup>b</sup>
Dairy fat (g)	0 ± 4	0	0	7 ± 6	23 ± 14
Plant oil (g)	21 ± 8 <sup>b</sup>	20 ± 13 <sup>b</sup>	24 ± 14 <sup>b</sup>	26 ± 27 <sup>b</sup>	11 ± 7 <sup>a</sup>
Plant protein (g)	59 ± 13 <sup>b</sup>	38 ± 8 <sup>a</sup>	59 ± 18 <sup>b</sup>	35 ± 9 <sup>a</sup>	35 ± 10 <sup>a</sup>
<i>B</i> -carotene (RE)	420 ± 348 <sup>c</sup>	209 ± 259 <sup>b</sup>	610 ± 378 <sup>d</sup>	43 ± 68 <sup>a</sup>	19 ± 24 <sup>a</sup>
Retinol (RE)	14 ± 32 <sup>a</sup>	36 ± 39 <sup>a</sup>	11 ± 22 <sup>a</sup>	59 ± 96 <sup>a</sup>	507 ± 760 <sup>b</sup>
Copper (mg)	2.3 ± 0.5 <sup>b</sup>	1.7 ± 0.6 <sup>a</sup>	2.9 ± 0.8 <sup>c</sup>	1.5 ± 0.5 <sup>a</sup>	2.8 ± 1.0 <sup>c</sup>

\*Values are county means ± SD. Significantly different values at  $P < 0.05$  within rows by the Tukey-Kramer test have different superscripts. Dairy fat was not tested due to limited data.

## INTRODUCTION

Assessment of vitamin A status is a critical though complex task for nutritionists. Two forms of the vitamin are generally consumed: preformed vitamin A (largely retinyl esters) from animal sources and the provitamin A carotenoids from plant sources. Homeostatic control of plasma retinol concentrations limits the utility of plasma retinol as a general indicator of vitamin A status. Uncertainty about the mechanism and extent of conversion of carotenoids, particularly *B*-carotene, and the association of carotenoids with circulating lipids<sup>1,2</sup> limits the interpretation of plasma *B*-carotene concentrations in relation to vitamin A status.<sup>3</sup> Disease states, lipid intake, and seasonal variations in carotenoid consumption also affect plasma vitamin A concentrations.<sup>4,5</sup> Acute-phase response to infection or trauma, characterized by inflammation and a rise in blood concentrations of C-reactive protein (CRP), commonly affect plasma nutrient concentrations.<sup>6</sup>

In studies largely with American, European, and Japanese subjects, plasma or serum retinol concentrations have been reported to be generally higher in men than women and are positively correlated with alcohol intake, body mass index (BMI), serum triacylglycerol, serum cholesterol, and smoking.<sup>1,2,7,8</sup> Except occasionally for supplement users, the plasma concentration of retinol is not correlated with intake of either preformed vitamin A or provitamin A carotenoids.<sup>1</sup> Plasma *B*-carotene concentrations are also positively correlated with plasma cholesterol.<sup>1,2</sup> In contrast with plasma retinol, plasma *B*-carotene has been reported to be negatively correlated with smoking, plasma triacylglycerol, BMI, and alcohol consumption.<sup>1,9,10</sup> Plasma *B*-carotene concentrations are generally higher in women and are correlated with the intake of carotenoid-rich foods and specifically with *B*-carotene intake.<sup>1</sup>

Five of the 65 counties originally surveyed in a 1983 ecological study in rural China<sup>11</sup> were resurveyed in 1989 to study the nutritional causes of osteoporosis in women.<sup>12,13</sup> The wide range of dietary and lifestyle patterns among these counties made these subjects an ideal group in which to study differing vitamin A nutriture from plant and animal sources. We also report on the physiologic, dietary, and lifestyle factors that are correlates of

plasma vitamin A among middle-aged women in these five rural areas of China.

## SUBJECTS AND METHODS

### Sampling Procedures

Details of the field survey including subject selection, a dietary survey, and a health and lifestyle questionnaire have been described previously.<sup>12,13</sup> Briefly, 5 counties were selected from the original 65 that participated in the 1983 ecological survey.<sup>11</sup> The county designations, CD, LC, SB, WA, and YA, were the designations in the original study. These sites were chosen to represent a wide variety of dietary patterns that are summarized in Table I. In each county about 150 women between the ages of 32 and 75 were randomly chosen. For this present study a subset of 404 middle-aged women were selected. Excluded were women more than 2 y postmenopausal. Included in the study were 293 women who reported themselves to be premenopausal ranging in age from 32 to 64, 63 individuals who reported being 0–2 y postmenopausal, and 48 individuals with incomplete reproductive information, 20 of whom were over 47 y, the average age of menopause in these counties. The survey was conducted between September and November 1989.

A detailed 3-d dietary survey was conducted by food weighing on each subject.<sup>12</sup> A general physical exam was conducted. Weights and heights were recorded and BMIs were calculated. An overnight 12-h urine sample was collected. A fasting blood sample was drawn and plasma was prepared. Plasma and urine samples were shipped on dry ice to Cornell University for analysis. A questionnaire on dietary, reproductive, smoking, and drinking habits was administered. Packs of cigarettes smoked and grams of alcohol consumed per week were recorded. Plasma and urinary cotinine were measured as previously described.<sup>13</sup> Smokers included the combination of women who either reported smoking or who had high concentrations of plasma cotinine (> 10 ng/mL) or urinary cotinine (> 100 mg/12 h). The smokers of county WA were detected by their cotinine concentrations only and were

possibly snuff users.<sup>11</sup> Drinkers were defined as women who reported any alcohol consumption.

The survey protocol was approved by the Human Subject Committee of Cornell University and the Chinese Academy of Preventive Medicine. The experimental protocol was fully explained to the subjects and their signed approval was obtained at the beginning of the survey.

#### Plasma Analyses

Plasma samples were stored frozen at  $-80^{\circ}\text{C}$  for 4–6 y and were defrosted for analysis. Others have shown that storage of plasma for 5 y at  $-70^{\circ}\text{C}$  is not detrimental to retinol and the carotenoids.<sup>14</sup> Plasma retinol was measured by modifications of the method of Driskall et al.<sup>15</sup> Internal standard (retinol acetate) in 600  $\mu\text{L}$  ethanol was added to a 100- $\mu\text{L}$  sample of plasma and 500  $\mu\text{L}$  water and was extracted with 900  $\mu\text{L}$  hexane. The hexane was evaporated and the sample resuspended with 75  $\mu\text{L}$  ethanol and then 225  $\mu\text{L}$  methanol. Duplicate 100- $\mu\text{L}$  injections were made with a Perkin-Elmer ISS-100 model autosampler (Norwalk, CT) onto a model 334 (Beckman Instruments, Cupertino, CA) high-pressure liquid chromatograph (HPLC) with an Ultramex 5- $\mu\text{m}$  C-18 4.6 X 250 mm column (Phenomenex, Inc., Torrance, CA). The mobile phase was methanol with a flow rate of 1.3 mL/min. A column heater (Timberline, Inc., Boulder, CO) maintained the column at a constant  $30^{\circ}\text{C}$ . The eluant was monitored at 300 nm with a Perkin-Elmer model LC-95 detector. Retinol eluted at 3.1 min and retinol acetate at 4.2 min. Plasma concentrations of retinol were calculated by comparison with peak heights of authentic standards and were adjusted for the recovery of the internal standard. The National Institute of Standards and Technology standard reference material 968a (fat soluble vitamins in human serum) was analyzed by this method and the accuracy of the method confirmed. Within the physiologic range the coefficient of variation of the method was 10%.

Plasma  $\alpha$ -carotene and  $\beta$ -carotene were quantified by modification of the preceding procedure for retinol. The internal standard was echinonone and was a gift of Roche, Inc., Nutley, NJ. The sample preparation procedure and the HPLC equipment were the same as for retinol. The HPLC column was an Ultramex 3- $\mu\text{m}$  C-18 4.6 X 250 mm column with titanium frits. The mobile phase was dichloromethane/acetonitrile/methanol, 20/45/35, with 0.05 mol/L ammonium acetate and 0.05% triethylamine. The flow rate was 1.0 mL/min. The detector was set at 450 nm.  $\alpha$ -Carotene eluted at 10.2 min,  $\beta$ -carotene at 10.6 min, a small *cis*  $\beta$ -carotene peak at 11.1 min, and the internal standard at 6.4 min. Verification of the accuracy of the method was similar to retinol. When analyzing the standard reference material 968a, recovery of  $\beta$ -carotene averaged 85%. Within the physiologic range of concentrations the coefficient of variation for  $\beta$ -carotene was 11%.

Plasma albumin was measured with Sigma Chemical kit 631 (St. Louis, MO) using the bromocresol green binding principle. Each sample was measured in triplicate and the median value used in the calculations. Analysis of a certified sample (Sigma M3366) verified the accuracy of the assay. The coefficient of variation was 5%. Plasma cholesterol was measured with Sigma kit 352, by the cholesterol oxidase-peroxidase method, with verification using Sigma certified sample C7921. The coefficient of variation was 6%.

Plasma copper was measured by the method of Abe et al.,<sup>16</sup> which involved selective and quantitative binding of  $\text{Cu}^{2+}$  to the chromogen, 4-(3,5-dibromo-2-pyridylazo)-*N*-ethyl-*N*-sulfopropylaniline. The National Institute of Standards and Technology standard reference material 1598 (inorganic constituents in bovine serum) was found by this method to be 34% above the certified value. The coefficient of variation in the physiologic range was 17%.

Retinol-binding protein (RBP), apolipoprotein B (ApoB), ceruloplasmin, and CRP were measured by automated nephelometry using a Behring Diagnostics model BN100 (Somerville, NJ). The accuracy of these methods was confirmed using Behring N/T CRP Control Serum, N/T Protein Control Serum, Apolipoprotein Control Serum CHD, and BioRad Liquichek (Hercules, CA). The coefficient of variations were 6% for RBP, 3% for ceruloplasmin, and 5% for ApoB. Over a wide range of CRP titers the coefficient of variation was about 10%.

#### Statistics

The data were analyzed using Systat 5.03 statistical software (Evanston, IL). County means were compared by the Tukey-Kramer post-hoc multiple comparisons test. The tabulation of subjects was tested for significance by Chi-squared. Linear regression models were always performed with county of residence as a variable.

The farming counties (CD, LC, and SB) exhibited similar lifestyles, diets, and particularly dietary vitamin A sources. Data from these counties were combined for multiple linear models in the assessment of nutrient status. Plasma  $\alpha$ - and  $\beta$ -carotene values were log transformed. Both plasma carotenenes exhibited skewed distributions that were substantially normalized by this operation. In models with menopausal status as a variable, subjects with no indicated menopausal status (48 subjects) and women who declared themselves to be premenopausal but were over 55 y old (4 subjects) were excluded from the analysis.

#### RESULTS

The principal diets of the five survey counties are described in Table I. Total energy intake in MJ (0.0042 MJ/kcal) generally reflected physical work activities. Energy intakes were highest in the farming counties, in Shanxi province (CD), Fijian province (LC), and Sichuan province (SB). Intakes were lower and more comparable to average American levels in the pastoral survey areas in Xinjiang province (WA) and Neimongol province (YA). Differing intakes of meat and dairy fat reflect the wide diversity of dietary patterns in these counties. Meat and dairy protein intakes from these sources closely resembled fat intake (data not shown).

Vegetable oil (usually rapeseed oil) was widely used, though lower in Neimongol, and was not correlated to the consumption of other plant products. Plant protein consumption was the inverse of meat consumption. Carbohydrate consumption was high in the farming areas, especially Sichuan, where plant-based energy intake from protein and carbohydrates comprised 90% of total energy consumption (compared with 56% in the USA).

Total vitamin A intake was low to moderate across the survey counties.  $\beta$ -carotene was the predominant form of dietary vitamin A in the farming areas, 94% of the total retinol equivalents (RE), although mean consumption was equivalent to only 55% of the Chinese and American recommended daily allowances (RDA) of 800 RE/d. Preformed vitamin A from dairy sources was the predominant form in county YA (80% of the total RE). County WA, with low intakes both of dairy and vegetable foods, was low in both forms of the vitamin with a mean daily consumption of only 102 RE. In the five counties, the major dietary sources of provitamin-A carotenoids were carrots, fresh and pickled Chinese cabbage, fresh and dried sweet potatoes, and dried chili peppers. Dietary preformed vitamin A was most often provided by goat liver, milk, and milk products (data not shown).

Table II summarizes plasma constituents and Table III summarizes other nutritional, disease, and lifestyle characteristics of the women. Plasma retinol concentrations were generally in the expected range for middle-aged women, though average concentrations were lower in counties SB and WA where the incidence of marginally low plasma retinol (defined as a plasma retinol

TABLE II.

	CONCENTRATIONS OF SELECTED PLASMA CONSTITUENTS OF CHINESE WOMEN IN FIVE RURAL COUNTIES*				
	CD	County			
		LC	SB	WA	YA
Retinol ( $\mu$ ,mol/L)	1.34 $\pm$ 0.30 <sup>ab</sup>	1.56 $\pm$ 0.38 <sup>c</sup>	1.27 $\pm$ 0.33 <sup>ab</sup>	1.22 $\pm$ 0.34 <sup>a</sup>	1.38 $\pm$ 0.31 <sup>b</sup>
RBP (mg/L)	28 $\pm$ 7 <sup>b</sup>	31 $\pm$ 8 <sup>b</sup>	26 $\pm$ 7 <sup>a</sup>	23 $\pm$ 7 <sup>a</sup>	29 $\pm$ 7 <sup>b</sup>
<i>B</i> -carotene ( $\mu$ ,mol/L)	0.28 $\pm$ 0.15 <sup>bc</sup>	0.66 $\pm$ 0.54 <sup>d</sup>	0.40 $\pm$ 0.24 <sup>c</sup>	0.16 $\pm$ 0.23 <sup>ab</sup>	0.13 $\pm$ 0.07 <sup>a</sup>
Albumin (g/L)	52 $\pm$ 6 <sup>c</sup>	51 $\pm$ 5 <sup>c</sup>	49 $\pm$ 5 <sup>b</sup>	48 $\pm$ 5 <sup>ab</sup>	46 $\pm$ 5 <sup>a</sup>
Cholesterol (mmol/L)	3.7 $\pm$ 0.9 <sup>a</sup>	4.3 $\pm$ 1.0 <sup>b</sup>	3.8 $\pm$ 0.7 <sup>a</sup>	3.8 $\pm$ 0.8 <sup>a</sup>	4.6 $\pm$ 0.9 <sup>b</sup>
ApoB (mg/L)	593 $\pm$ 137 <sup>ac</sup>	655 $\pm$ 187 <sup>c</sup>	548 $\pm$ 137 <sup>a</sup>	578 $\pm$ 194 <sup>ab</sup>	632 $\pm$ 156 <sup>bc</sup>
Copper ( $\mu$ ,mol/L)	15 $\pm$ 4 <sup>ab</sup>	14 $\pm$ 4 <sup>a</sup>	15 $\pm$ 6 <sup>ab</sup>	16 $\pm$ 5 <sup>b</sup>	16 $\pm$ 5 <sup>b</sup>
Ceruloplasmin (mg/L)	338 $\pm$ 78 <sup>a</sup>	347 $\pm$ 120 <sup>a</sup>	314 $\pm$ 82 <sup>a</sup>	427 $\pm$ 131 <sup>b</sup>	399 $\pm$ 119 <sup>b</sup>
CRP (mg/L)	8 $\pm$ 23 <sup>a</sup>	10 $\pm$ 27 <sup>a</sup>	10 $\pm$ 38 <sup>a</sup>	31 $\pm$ 76 <sup>b</sup>	5 $\pm$ 9 <sup>a</sup>

\* Values are means  $\pm$  SD. Significantly different values at  $P < 0.05$  within rows by the Tukey-Kramer test have different superscripts. ApoB, apolipoprotein B; CRP, C-reactive protein; RBP, retinol-binding protein.

value  $< 0.7 \mu$ ,mol/L) was elevated. Plasma *B*-carotene concentrations reflected Western and Japanese values for counties LC and SB, whereas the remaining counties were markedly lower particularly in the pastoral counties WA and YA, where *B*-carotene consumption was very low.

Plasma cholesterol was generally below 5 mmol/L (193 mg/dL). The percent of subjects with values over 5 mmol/L were highest in county YA and lowest in county SB. CRP showed considerable variation between counties. Values were relatively high in county WA compared with other counties. Distributions of CRP within counties were highly skewed with many undetectable concentrations and a few individuals with values over 50 mg/L. County average concentrations of albumin and copper were rather uniform among these counties despite wide variation among individuals. Ceruloplasmin concentrations paralleled cholesterol and ApoB concentrations across counties.

The consumption of alcoholic beverages was low in rural China and only 1 subject reported smoking more than 1 pack of cigarettes per day. The subjects were relatively lean compared with American women. The percent of subjects classified as overweight, with BMI over 25, varied from a low of 1% in county SB to a high of 27% in county CD.

High concentrations of CRP corresponded to an increased incidence of marginally low plasma retinol concentrations (Table IV). One-third of the women with CRP concentrations greater than 100 had values of plasma retinol below  $0.7 \mu$ ,mol/L, whereas only 1% of women with normal concentrations of CRP had low plasma retinol concentrations. Ceruloplasmin, a positive acute-phase reactant, and plasma *B*-carotene were also significantly different in higher CRP groups (Table V). Plasma RBP and albumin were only slightly decreased in the high-CRP group (data not shown). Among subjects with marginally low plasma retinol concentra-

TABLE III.

	LIFESTYLE AND PHYSIOLOGIC CHARACTERISTICS OF CHINESE WOMEN IN FIVE RURAL COUNTIES				
	CD	County			
		LC	SB	WA	YA
Age* (y)	43 $\pm$ 5	43 $\pm$ 6	44 $\pm$ 7	43 $\pm$ 6	43 $\pm$ 6
Drinkers (%)	0	2	21	0	3
Alcohol intake† (g/wk)	—	52	55	—	217
Smokers (%)	34	0	14	9	42
Smoking (pk/wk)†	1.7	—	2.0	—	3.5
High cholesterol (% $> 5$ mmol/L)	8	15	4	10	28
High CRP (% $\geq 50$ mg/L)	3	5	5	13	0
BMI‡ (kg/m <sup>2</sup> )	23.6 $\pm$ 3.5 <sup>c</sup>	21.3 $\pm$ 3.3 <sup>ab</sup>	20.2 $\pm$ 2.1 <sup>a</sup>	22.8 $\pm$ 3.6 <sup>c</sup>	22.4 $\pm$ 2.9 <sup>bc</sup>
Overweight (% $> 25$ kg/m <sup>2</sup> )	27	14	1	22	19
Low plasma retinol (% $< 0.7 \mu$ ,mol/L)	0	1	5	6	1

\* Mean  $\pm$  SD. Average age was not significantly different between counties.

† Alcohol intake and smoking are median values among drinkers only or smokers only.

‡ Mean  $\pm$  SD. BMI values with different superscripts are statistically different at  $P < 0.05$  using the Tukey-Kramer test. BMI, body mass index; CRP, C-reactive protein.

TABLE IV.

C-REACTIVE PROTEIN (CRP) AND VITAMIN A DEFICIENCY		
Plasma CRP (mg/L)	No. subjects	% Subjects with plasma retinol < 0.7 $\mu$ ,mol/L
0	198	1*
1-100	152	4
≥100	9	33

\* Differences in percentage incidence are significant by Chi-squared to  $P < 0.001$ .

tions, CRP concentrations are also significantly elevated ( $P < 0.001$ , data not shown) and plasma retinol and CRP concentrations are inversely correlated ( $r = -0.15$ ,  $P < 0.001$ ).

Plasma retinol and retinol-binding protein-carotene concentrations were compared with several diet and lifestyle indicators (Table VI). Only data from the three farming counties (CD, LC, and SB) were utilized and controlled for in these linear models. The predominant source of vitamin A in these counties was  $\beta$ -carotene and the levels of animal products (including preformed vitamin A) in the diet were low in all three counties. Plasma cholesterol was positively correlated with plasma retinol,  $\beta$ -carotene, and  $\alpha$ -carotene. Controlling for age, plasma retinol was higher in women who were 1-2 y postmenopausal compared with those who were premenopausal, whereas  $\alpha$ - and  $\beta$ -carotene were lower in 1-2 y postmenopausal individuals. After adjusting for plasma cholesterol and survey county, plasma retinol did not correlate with smoking, drinking, or age, or with the consumption of preformed retinol or  $\beta$ -carotene (data not shown).

A limited number of linear models were examined with regard to correlates of plasma retinol (Table VII, Table VIII). Among the farming counties plasma retinol was predicted by a linear combination of survey county, plasma  $\beta$ -carotene, plasma cholesterol, plasma copper, menopausal status, and age (Table VII). Plasma copper concentration was itself correlated with the intake of copper ( $P = 0.007$ ). Plasma  $\beta$ -carotene, in the farming counties, was predicted by a linear combination of county, plasma chole-

TABLE V.

C-REACTIVE PROTEIN (CRP) AND OTHER ACUTE-PHASE REACTANTS*			
Plasma CRP (mg/L)	Plasma retinol ( $\mu$ ,mol/L)	Ceruloplasmin (mg/L)	Plasma $\beta$ -carotene ( $\mu$ ,mol/L)
0	1.38 <sup>a</sup>	332 <sup>a</sup>	0.28 <sup>a</sup>
1-100	1.33 <sup>ab</sup>	393 <sup>b</sup>	0.17 <sup>b</sup>
≥100	1.11 <sup>b</sup>	517 <sup>c</sup>	0.07 <sup>c</sup>
ANOVA	$P = 0.050$	$P < 0.001$	$P < 0.001$

\* Statistics for plasma  $\beta$ -carotene were performed using logarithm-transformed values. Values shown are untransformed group means. Values within columns with different letter superscripts were significantly different at  $P < 0.05$  using the Tukey test. The number of subjects in each CRP subgroup is the same as for Table IV.

TABLE VI.

CORRELATES OF PLASMA RETINOL, $\beta$ -CAROTENE, AND $\alpha$ -CAROTENE IN FARMING COUNTIES			
Variable	Retinol* ( $\mu$ ,mol/L)	$\beta$ -Carotene* ( $\mu$ ,mol/L)	$\alpha$ -Carotene* ( $\mu$ ,mol/L)
Plasma cholesterol (mmol/L)	+0.093 <sup>†</sup> $P = 0.001$	+0.164 $P = 0.002$	+0.162 $P < 0.001$
Postmenopausal <sup>‡</sup> (no = 0, yes = 1)	+0.165 $P = 0.028$	-0.243 $P = 0.001$	-0.120 $P = 0.031$
Plasma retinol ( $\mu$ ,mol/L)	—	+0.251 $P = 0.061$	+0.277 $P = 0.006$

\* Logarithmically transformed data were used for these variables.

<sup>†</sup> Values are linear model coefficients with only county and the row variable predicting the column variable. The associated partial  $P$  value is for the row variable in that model.

<sup>‡</sup> Age was also included in the linear model with the dichotomous variable for menopause.

terol, plasma CRP, menopausal status, and age. Either before or after controlling for these independent predictors of plasma  $\beta$ -carotene, no measurement of nutrient intake, including dietary fiber, lipid, vitamin C, or  $\beta$ -carotene was significantly correlated with plasma  $\beta$ -carotene (data not shown).

Among the farming counties, plasma cholesterol was predicted by county, age, BMI, lipid intake, and animal protein consumption. The intake of lipid from either animal or plant sources showed similar moderately inverse effects in this model (data not shown) as did total lipid consumption.

In contrast to the farming counties, among the pastoral counties (WA and YA) with very low  $\beta$ -carotene intake and relatively high consumption of animal products, plasma retinol was not correlated with plasma  $\beta$ -carotene nor with menopausal status and was negatively correlated with plasma CRP concentration (Table VIII).

## DISCUSSION

Vitamin A consumption among this female cohort in rural China was relatively low compared with the reported female American and European average daily consumption of 400 RE of  $\beta$ -carotene and 900 RE of preformed vitamin A.<sup>17,18</sup> Mean total vitamin A consumption was about 50% of the RDA for all the study areas together. Individuals in county WA consumed only 102 RE/d of total vitamin A (13% of the RDA) with only 59 RE/d from preformed vitamin A. Surprisingly, only 6% of the WA subjects had marginally low plasma retinol concentrations of less than 0.7  $\mu$ ,mol/L, and plasma retinol was not correlated with intake of either preformed or provitamin A. The relatively high consumption of total lipids compared with the other counties, at 25% of energy, may facilitate vitamin A absorption in this county.

This survey was conducted during the harvest season of September to November. This was a cross-sectional survey and one concern might be that food consumption in the season of the survey may not reflect year-round consumption of vitamin-A-rich foods. From the 1983 ecologic study of food consumption in 65 rural counties<sup>11,19</sup> and from a similar study in 1989 (unpublished), the average year-round consumption of carotenoid-rich foods was a little less than one serving per day. These foods were generally staple foods available year-round and included dried and fresh sweet potato, carrots, and pickled Chinese cabbage. Preformed vitamin A, largely available from liver and dairy products, was

TABLE VII.

LINEAR MODELS OF DETERMINANTS OF PLASMA RETINOL IN FARMING COUNTIES ONLY*				
Independent variables	Univariate		Multivariate	
	<i>r</i>	<i>P</i>	<i>B</i>	<i>P</i>
Dependent variable: Plasma retinol ( $\mu$ ,mol/L)				
County	—	<0.001	—	0.001
Plasma <i>B</i> -carotene†	0.22	0.002	0.064	0.084
Plasma cholesterol	0.31	<0.001	0.056	0.044
Plasma copper	0.19	0.007	0.016	<0.001
Menopause‡	0.08	0.225	0.155	0.035
Age	-0.10	0.150	-0.012	0.007
$R^2 = 0.22$ ( $P < 0.001$ )				
Dependent variable: Plasma <i>B</i> -carotene (log of $\mu$ ,mol/L)				
County	—	<0.001	—	<0.001
Plasma cholesterol	0.29	<0.001	0.186	<0.001
Plasma CRP§	-0.23	0.001	-0.208	<0.001
Menopause‡	-0.19	0.006	-0.247	<0.001
Age	-0.12	0.084	0.004	0.592
$R^2 = 0.33$ ( $P < 0.001$ )				
Dependent variable: Plasma cholesterol (mmol/L)				
County	—	<0.001	—	0.082
Age	0.10	0.150	0.017	0.104
Body mass index	0.04	0.530	0.035	0.073
Dietary lipid	-0.03	0.644	-0.009	0.047
Dietary animal protein	0.28	<0.001	0.032	0.003
$R^2 = 0.14$ ( $P = 0.05$ )				

\* Approximately 222 subjects in each model.

† Logarithm transformed.

‡ pre = 0, post = 1.

§ Square root transformed.

CRP, C-reactive protein.

also generally available year-round but in a limited geographic area. Therefore, there appears to be very little seasonality bias to the vitamin A intake data. The adequacy of carotenoids as sources of vitamin A has been questioned recently.<sup>20,21</sup> The indications from the farming counties of this survey were that in these counties where preformed vitamin A intake was extremely low, carotenoid-rich foods provided adequate sources of vitamin A.

Subjects with moderate or high concentrations of CRP were significantly more likely to have marginally low concentrations of plasma retinol. In response to infection or other trauma, plasma concentrations of a number of proteins and other constituents are altered within a day or two. Previous studies have shown that acute-phase response leads to decreases in plasma concentrations of carotenes, albumin, retinol, RBP, copper, and cholesterol and increases in ceruloplasmin.<sup>6,22</sup> We confirmed a negative correlation between CRP and plasma *B*-carotene and retinol, and a positive correlation with ceruloplasmin, but fail to find a significant association with cholesterol, ApoB, albumin, RBP, or copper. These results suggest that the acute-phase response seen in some subjects may have caused a decrease in plasma levels of retinol and *B*-carotene and further suggests that apparent vitamin A deficiencies in rural China may be due in part to the acute-phase response and not low vitamin A intake. However, a role for low vitamin A status in causing infection and inflammation cannot be ruled out based on this cross-sectional study.

There was no correlation between *B*-carotene consumption and

TABLE VIII.

LINEAR MODELS OF DETERMINANTS OF PLASMA RETINOL IN PASTORAL COUNTIES ONLY*				
Independent variables	Univariate		Multivariate	
	<i>r</i>	<i>P</i>	<i>B</i>	<i>P</i>
Dependent variable: Plasma retinol ( $\mu$ ,mol/L)				
County	—	0.002	—	0.251
Plasma cholesterol	0.36	<0.001	0.107	0.002
Plasma copper	0.13	0.098	0.010	0.068
Plasma CRP†	-0.27	0.001	-0.019	0.020
$R^2 = 0.20$ ( $P = 0.001$ )				

\*  $N = 141$ .

† Square root transformed.

plasma *B*-carotene concentrations. This is in contrast to many previous reports with subjects from more economically developed regions of the world in which positive correlations have been reported before and after controlling for confounders such as sex, age, and plasma cholesterol.<sup>23,24</sup> In the present study no measured dietary parameters, including dietary fiber, lipids, retinol, copper, or vitamin C, were significantly correlated with plasma *B*-carotene. This unusual finding may be related to the cross-sectional nature of this study. However, cross-sectional studies in the United States that demonstrated a strong correlation ( $r = 0.41$ – $0.51$ ) between intakes and plasma levels of *B*-carotene used semiquantitative food frequency questionnaires and 7-d food records.<sup>23,24</sup> This lack of correlation in rural China may also be due to intestinal conversion of dietary *B*-carotene to retinol. In populations for which the major source of vitamin A is *B*-carotene, the plasma *B*-carotene concentration, roughly approximating liver storage levels of *B*-carotene, might be expected to be lower in individuals with a higher intestinal conversion of *B*-carotene to retinol and hence a possibly more normal plasma retinol level. Conversely, the high correlation among women with high intakes of both preformed and provitamin A in the United States may reflect a lack of intestinal conversion of *B*-carotene to retinol.

Despite these limitations and complications, these results suggest that plasma *B*-carotene concentrations, in context of the dietary and lifestyle conditions in rural China, are influenced less by extrinsic factors, such as diet and lifestyle, and more by intrinsic factors, possibly including physiologic or pathologic factors affecting overall vitamin A status. What those intrinsic factors are is still unclear but our results suggest that they may include vitamin A requirements, hormonal factors related to menopause, factors affecting acute-phase response, and factors affecting lipid status as reflected by plasma cholesterol.

Our study was focused on premenopausal and recently menopausal women. After controlling for age and county of residence, we found that postmenopausal women had 10% higher plasma retinol and 40% lower *B*-carotene than premenopausal women. Adult women generally have lower plasma retinol and higher plasma *B*-carotene concentrations than men.<sup>17,18,25</sup> Our results suggest that within 2 y of menopause, the gender differences in plasma retinol and *B*-carotene disappear in a population dependent on carotenoids as their source of vitamin A.

The finding that plasma copper was positively and independently correlated with plasma retinol in this ecologic study has not been previously reported to our knowledge. In contrast, Tebi et al.<sup>26</sup> have reported an inverse correlation in elderly subjects.

Animal studies have shown that feeding diets deficient in copper leads to reduced retinol concentrations.<sup>27</sup> However, in human studies plasma copper is generally not correlated with copper intake.<sup>28</sup> In this study we found a correlation between the intake of plant sources of copper and plasma copper concentrations in the three farming counties. These results suggest that under the dietary and lifestyle conditions in rural China, plasma copper concentration may reflect intake and may affect plasma retinol in a manner similar to the animal studies<sup>27</sup> and in contrast to the previous human studies.<sup>26,28</sup>

Unlike most Westerners, most rural Chinese rely on carotenoids as their major dietary source of vitamin A. Various individual differences in physiologic factors, such as the efficiency of conversion of *B*-carotene to retinol, vitamin A requirements and metabolism, carotene mobilization in low-density lipoproteins,

and possibly heightened states of acute-phase response, may override differences in *B*-carotene intake as predictors of plasma *B*-carotene concentrations. Nonetheless, among these women, dietary *B*-carotene was an adequate source of vitamin A nutrition even in the context of relatively high fiber intake and low fat intake relative to Western populations. Further research is needed to determine the extent to which other environmental and dietary factors or physiologic and pathologic factors may play a role in vitamin A nutrition in rural China.

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