Nutritional Status of Vitamin A, E, C, B_1, B_2, B_6, Nicotinic Acid, B_{12}, Folate, and β-Carotene in Young Women

—Relationships between Blood Concentrations and Dietary Intakes—

若年女性のビタミン栄養状態（ビタミンA, E, C, B_1, B_2, B_6, ニコチン酸, B_{12}, 葉酸およびβ-カロチン）について

—血中濃度と摂取量の関係—

A Dissertation Presented to
the Division of Health Sciences
the Graduate School of Kagawa Nutrition University
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March 16, 2000

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Summary

To investigate the vitamin status of young Japanese women, dietary intakes of vitamin A, E, C, B_1, B_2, B_6, B_{12}, niacin, folate, and β-carotene were assessed by a 3-day weighed food record in 150 female students aged 21－22. Whole blood levels of vitamin B_1, B_2, and nicotinic acid, and serum levels of retinol, α-tocopherol, vitamin B_6, and β-carotene were determined by HPLC. Vitamin B_{12} and folate in serum were measured by chemiluminescence immunoassay, and serum ascorbic acid was done by dinitrophenylhydrazine method. When the RDAs for the Japanese (6th revision) were applied, 46.7% of the females were sufficient intake for vitamin A, 28.7% for E, 68.0% for B_1, 78.0% for B_2, 54.7% for B_6, 97.3% for niacin, 76.0% for B_{12}, 34.0% for folate, and 54.0% for C. Fifty-nine percent of total vitamin A (μgRE) intakes were derived from β-carotene. The mean±SD of energy intakes was 1,572±315kcal, which was equivalent to the energy requirement for the level "Ⅰ(low)" of physical activity. Significant correlations among intakes of energy and all these vitamins were found. Whole blood vitamin B_2, serum folate and ascorbic acid in the females with corresponding vitamin intakes above the RDA were significantly higher than in those with intakes below the RDA. There were significant correlations between blood vitamin levels and vitamin intakes in vitamin B_{12} (r=0.185), folate (r=0.255), vitamin C (r=0.272), and β-carotene (r=0.319). Mean blood levels of folate, ascorbic acid, vitamin B_2, B_{12}, and β-carotene were higher in the highest quartile of intake than in the lowest. The 95% confidence intervals of blood vitamin levels obtained from the females with sufficient vitamin intakes were nearly equal to those obtained from all subjects. Only a few females (0.7－4.7%) had their blood vitamin levels below the lower limits. Serum α-tocopherol levels were significantly correlated with serum levels of retinol, β-carotene, and ascorbic acid.

This data suggested that further studies might be needed to establish the more appropriate RDAs of vitamin E and folate for young women, judging from the relationships between blood vitamin levels and dietary intakes.

Introduction

The diversification of lifestyle has caused eating habits to change, and increase the prevalence of anorexia nervosa and bulimia nervosa, especially in young women, because of a desire to slim down, even though there has been a flood of information on nutrition and supplements (*1*). Recommended allowances of every vitamin are determined by the 6th revision of the Recommended Dietary Allowances (RDAs) for Japanese (*2*), whereas in previous revision (*3*), recommended allowance of vitamin A, B_1, B_2, C, D, and niacin, and safe and adequate daily dietary intake of vitamin E were indicated. There have been few reports on vitamin nutritional status in Japanese, except for the above mentioned seven vitamins (*4*).

The dietary vitamin intakes and blood levels are generally used as indispensable indicators of vitamin nutrition status. Accordingly, in this study, intake and blood level of vitamin A, E, C, B_1, B_2, B_6, B_{12}, nicotinic acid, folate, and β-carotene were assessed and relationships between intake and blood vitamins were investigated in Japanese female students.

Methods

Subjects. Subjects were 150 female students of our university, aged 21-22, who were not taking vitamin supplements. The study was carried out in April 1996-1997. The mean height, weight, and body mass index (BMI; weight(kg)/height(m)^2) of subjects were 158.4±5.0 cm, 52.6±6.3 kg, 20.9±2.2. Informed written consent was obtained from all subjects, later the purpose, significance, and the protocols of the study were fully explained. All study procedures were approved by Medical Ethics Committee of Kagawa Nutrition University.

Nutrition intake. The subjects provided a successive 3-day weighed food record immediately before the day of collection of blood samples. Nutrients intakes were calculated, based on the Standard Tables of Food Composition in Japan (*5*). Since available food composition tables in Japan did not permit the calculation of folate, intakes of folate were obtained from "Bowes and Church's Food Values of Portions Commonly Used (*6*)." The amount of the niacin intake from tryptophan ; 1mg niacin as equivalent 60mg tryptophan, and niacin intake from food was referred to as "niacin equivalent." Unregistered items in the food tables were substituted by the mean of existing items in the same food groups. Dietary intakes were compared to the 6th revision of Japanese Recommended Dietary Allowances (RDAs) for female aged 18-29 with physical activities of level Ⅲ (*2*) which were also nearly equal to those of level Ⅱ in previous revision (*3*).

Determination of blood vitamins. Blood samples were collected into evacuated tubes with and without EDTA-2K before breakfast. Serum was separated from the non-EDTA containing blood for analyses of retinol, β-carotene, α-tocopherol, total vitamin B_6, folate, vitamin B_{12}, and ascorbic acid. Whole blood with anticoagulant was used to the total vitamin B_1, total vitamin B_2, and total nicotinic acid. Vitamin B_1 levels were also measured in serum of some subjects (n=80). Blood samples were stored at −30°C until they were analyzed for vitamin concentrations except for ascorbic acid which was

analyzed immediately.

Concentrations of serum retinol and α-tocopherol were simultaneously determined by HPLC (*7*). Retinol and α-tocopherol in serum were extracted with hexane. The column used was a SHIM-PACK FLC-SIL (4.6mm × 50mm, Shimadzu, Japan) with a mobile phase of n-hexane:2-propanol (99.1:0.9) at a flow rate of 0.8mL/min. Detection was at 295nm.

Serum β-carotene was determined by reverse-phase HPLC. β-carotene in serum was extracted using a 200μL sample, 1.0mL water, and 1.0mL ethanol. After the addition to 5mL n-hexane, the mixture was vortexed and then centrifuged. The hexane layer was evaporated to dryness under nitrogen at 30°C. The residue was redissolved in 150μL ethanol and 50μL aliquot was injected into the HPLC column. The column was Inertsil ODS-2 (4.6mm × 150mm, GL Science, K.K, Japan). The mobile phase was methanol: acetonitril: dichloromethane (75:13:12), with 2% water (by vol) at a flow rate of 1.5mL/min. β-carotene was detected at 453nm.

Total vitamin B_1 in whole blood and in serum was determined by HPLC with pre-column derivatization (*8*). The mixture of 0.5mL whole blood or serum, and 0.5mL 0.1M acetate buffer (pH4.5) were added to 1.2mL 0.4M(6.5%) TCA and shaken. After centrifugation, 1.0mL supernatant was brought up to pH4.5 by addition to 4M sodium acetate and added 50μL of 2% takadiastase and incubated at 37°C for 4h. Then the thiamin was derivatizated to the thiochrome by adding 0.1mL 10% cyanogen bromaide and 0.1mL 2M NaOH to 0.8mL of the sample extract. The 50μL derivatized samples was injected into the column. The column was TSK-gel G3000PWXL (7.8mm × 300mm, TOSOH, Japan). The mobile phase was 15% acetonitril in 90mM potassium phosphate buffer (pH8.6) at a flow rate of 1.0mL/min. The chromatographic effluent was monitored by fluorescence detector set at 365nm for excitation and at 430nm for emission.

Whole blood total vitamin B_2 was determined by HPLC method (*8*). To be brief, the mixture of 0.5mL whole blood and 3.5mL 0.1N H_2SO_4 was incubated

at 80°C for 15min then 1.0mL 10% TCA was added. After centrifugation, 1.0mL of the supernatant was added to 1.0mL 1N NaOH and laid under the light for 45min at room temperature for photolysis of vitamin B_2 to lumiflavin. Then the mixture was made acidic with 0.1mL of acetic acid and injected into HPLC column. The column was Inertsil ODS-2 (4.6mm × 150mm, GL Science, Japan). The mobile phase was 35% methanol in 10mM KH_2PO_4 at a flow rate of 0.5mL/min. Wave lengths of fluorescence detector were 445nm for excitation and 530nm for emission.

Serum total vitamin B_6 determination was performed by HPLC with post-column method (*9*). 100 μL of 9N $HClO_4$ was added to 1.0mL of serum, then after centrifugation, clear supernatant was injected into the column. The column was TSK-gel ODS120T (4.6mm × 250mm, TOSOH, Japan). The mobile phase was 75mM KH_2PO_4 buffer containing 75mM $NaClO_4$, 0.85% acetonitril, and 0.05% triethanolamine adjusted to pH 3.38 with concentrated $HClO_4$.The flow rate was 1.0mL/min. After post-column derivatization of pyridoxal phosphate with 250mM K_2HPO_4 buffer (pH11.7) containing 0.1% sodium disulfate at a flow rate of 0.1mL/min, column effluent was monitored by fluorescence detector set at 325nm for excitation and at 400nm for emission.

Total nicotinic acid in whole blood was determined by HPLC (*10*). For the extraction of nicotinic acid, 1.0mL of whole blood was added 0.4mL water and 0.4mL of 10N NaOH, then the mixture was heated at 100°C for 30min. After adjusted to pH2.7 with HCl and made up 3.0mL with water, and centrifugation, 0.5mL supernatant was loaded on Sep-Pak cartridges C_{18} (Waters), then the cartridge was flushed with water. Then nicotinic acid was eluted with 1.5mL of water:methanol (55:45). This eluate was injected into HPLC column. The column was Nucleosil C_{18} (4.6mm × 250mm, Nagel) and mobile phase was 10mM dioctylsulfosuccinate: methanol (145:105) with a flow rate of 0.8mL/min. Detection was at 254nm.

Serum folate and vitamin B_{12} levels were determined by the

chemiluminescence immunoassay using a kit (ACS:180 VB12/Folate, Bayer Medical).

Total ascorbic acid in serum was determined by a colorimetric method using 2,4-dinitrophenilhydradine (*11*).

Statistical analyses. All data were expressed as mean±standard deviation(SD). Means were compared using t tests or analysis of variance. All intakes and blood measures were positively skewed, requiring logarithmic transformations to normalize their distributions. Serum α-tocopherol values were correlated with serum total cholesterol ($r=0.480$, $p<0.001$). Therefore, absolute serum α-tocopherol values as well as the ratio of α-tocopherol to serum total cholesterol are presented where necessary. Mean blood vitamin levels are also presented across quartiles of vitamin intake. *P* values <0.05 were considered statistically significant.

Results

Mean daily vitamin intakes are presented in Table 1. Individual variations were observed, in regard to vitamin A intake. Subjects less than 50% took vitamin A above the RDA, while 4.7% (n=7) were over the permissible upper limit of intake, 1,500μgRE/day. The mean±SD of β-carotene intake was 2,240±1,623μg (373±270μgRE) and represented 59% of vitamin A. Concerning vitamin E and folate, mean intakes were below the RDAs, and the percentage of subjects with intakes above the RDAs was lower than that of other vitamins. Energy intake was extremely low (1,572±315kcal). Subjects consumed 58.5±13.6g/day of protein and 28.9±5.9% of fat energy ratio from 50.9±16.3g/day of fat. Only 2.0% (n=3) of the subjects were above the RDAs of overall nutrients assessed in this study, and 8.7% (n=13) were above the RDAs of these vitamins without energy. On the other hand, 2.7% (n=4) of subjects did not meet RDAs of overall nutrients.

Significant correlations were found among vitamins and energy intakes except between retinol and β-carotene, retinol and vitamin C, vitamin B_{12} and vitamin C (Table 2).

Blood vitamin levels are presented in Table 3. The means of whole blood vitamin B_2, serum folate, and serum ascorbic acid were significantly different between in the subjects with intake above RDA and ones with intake below RDA. However, no significant differences were detected in any other vitamins. Blood vitamin levels of subjects with all nutrient intakes above RDAs were higher than those of subjects with all nutrient intakes below RDAs, although the difference was not significant. Table 4 shows the 95% confidence intervals of blood vitamin levels obtained from the subjects with intake above the RDAs, which were little different from the values of all subjects.

The significant correlations between blood vitamin levels and vitamin intakes were observed for β-carotene ($r=0.319$, $p<0.001$), vitamin B_{12} ($r=0.185$, $p<0.02$), folate ($r=0.255$, $p<0.002$), and vitamin C ($r=0.272$, $p<0.001$). Vitamin E intake was significantly correlated with the ratio of serum α-

tocopherol/total cholesterol ($r=0.171$, $p<0.05$), but not with serum α-tocopherol.

Fig. 1 shows the mean blood vitamins by quartile of vitamin intakes. Mean concentrations of serum retinol, serum α-tocopherol, blood vitamin B_1, serum vitamin B_6, and blood nicotinic acid changed little across intake quartiles. Serum folate was 26.0% higher in the highest quartile of folate intake than in the lowest quartile of intake. Serum ascorbic acid showed difference 19.9% between extreme quartiles. For serum β-carotene, blood vitamin B_2, and serum vitamin B_{12}, the mean blood concentrations were 62.5%, 8.0% and 14.8% higher in the highest quartile of intake than in the lowest quartile of intake, respectively, although there was not significant trend over intake quartiles.

The correlation matrix between concentrations of blood vitamins is shown in Table 5. Serum α-tocopherol was significantly associated with antioxidant vitamins: retinol, β-carotene, and ascorbic acid.

To evaluate the relationship between a vitamin in serum and in erythrocyte, total vitamin B_1 in whole blood and in serum of some subjects (n=80) was determined. Erythrocyte total vitamin B_1 was calculated from hematocrit. The mean±SD of total vitamin B_1 levels was 11.8±4.9 nmol/L in serum, 110.9±28.9 nmol/L in whole blood, and 67.7±18.4 nmol/L in erythrocyte. No significant correlations were observed between any blood vitamin B_1 levels and intake of vitamin B_1. The ratio of serum vitamin B_1 to erythrocyte vitamin B_1 was 0.19±0.12, and was observed correlation coefficient for –0.427 ($p<0.001$) with whole blood vitamin B_1.

Discussion

The purpose of the present study was to evaluate the vitamin status of young women aged 21-22 by assessing their dietary intakes and measuring blood vitamin levels. Consciousness of obesity has an effect on the eating behavior of women, and about 60% of women have that consciousness and are interested in eating as well as slimming down (*1*). It was observed the mean energy intake of the female students was equivalent to the 6th revision RDA for level "Ⅰ(low)" of physical activity and if the energy intake was compared with the RDA for level "Ⅲ(moderate)" which was considered to be a desirable goal, it was very low. The energy requirement for the female students aged 21-22 with the level Ⅲ of physical activity in 6th revision, 2,050kcal/day, is nearly the same of that for those with the level Ⅱ in previous revision, 2,000kcal/day. Though energy intake was low, mean of BMI was a little higher than the estimated median BMI of a female aged 21 (20.4kg/m^2) for the year 2000 (*3*). No correlation between energy intake and BMI has reported (*12*). This could indicate low physical activities in female daily life.

Intake of vitamin A, B_1, B_2, and C has been reported in the National Nutrition Survey every year (*4*). For these vitamins except vitamin B_1, the results of mean intake agreed with the data of National Survey. Mean intake of vitamin B_1 was a little low, which was more than RDA. Recommended β-carotene allowance has not been estimated, and very few data of β-carotene intake for the Japanese have been reported (*13*). The author showed 59% of total vitamin A intake were from β-carotene (μgRE), and the percentage is similar in the other report (*14*). Vitamin E, B_6, B_{12}, and folate allowances were first established in 6th revision RDAs for the Japanese (*2*). The mean intakes of vitamin B_6 and B_{12} were above their RDAs, while vitamin E and folate were below their RDAs and only about 30% of subjects had intakes above the RDAs. The data on concentrations of folate in foods has not been yet included in the food composition table for the Japanese (*5*), and few data on folate intake of the Japanese have been reported. In a foreign report, folate intake in young

women aged 22 years is 188-200 μg/day, which is the same as in this study (*15*).

The significant correlations between intakes of energy and these vitamins were observed, consistent with the previous report (*12*). Therefore, it would be appropriate that on these nine vitamins, mean of the nutrient density (vitamin intake/1,000kcal energy intake) was sufficient to the vitamin RDA/1,000kcal energy RDA. The percentage of subjects with intake of vitamin B_1 above the allowance of 0.42mg/1,000kcal was 90.0%, and those with intake of vitamin B_2 above the allowance of 0.48mg/1,000kcal was 96.7%, and those with intake of niacin above the allowance of 6.3mgNE/1,000lcal was 100.0%. Intake of most vitamins related with one another. It seemed that the intake of vitamins from ordinary dietary meals without vitamin supplements was well-balanced. It would be necessary to increase moderate amounts of physical activities and suitable food intake so that the intake of vitamins would be increased. No correlation of intake between retinol and β-carotene, or retinol and vitamin C need be explained in that the main source of retinol was animal, and those of β-carotene and vitamin C were vegetable or fruit (*14*).

Serum β-carotene, vitamin B_{12}, folate, and ascorbic acid concentrations were significantly correlated to corresponding intakes, though the correlation coefficients were generally weaker than that previously reported by Jacques et al (*16*). Most correlations between intakes and biochemical indicators have been shown among subjects taking supplements or on experimental diets led to expand the range of nutrient intake (*17, 18*). However no correlation was observed between blood levels and intake of vitamin E, B_1, B_2, and nicotinic acid, mean blood concentration of these vitamins in the highest quartile of corresponding intake was higher than the lowest quartile. It could be suggested that the relationship between blood vitamin level and intake is not necessarily linear, especially with intake within the range of the general dietary meats, but the blood vitamin level plateau because the circulating concentration is influenced by many factors other than diet or under

homeostatic control (*16*).

The 95% confidence intervals of blood vitamin levels were obtained from the subjects with corresponding vitamin intake above the RDA, who were regarded as not deficiency. Whether intake vitamin was sufficient or not, the percentage of subjects with the blood vitamin levels below the lower limit of their 95% confidence intervals were few. The cut-off levels of serum β-carotene, vitamin B_{12}, folate, and ascorbic acid, which correlated with intake, were higher than those by Tietz (*19*). As for serum α-tocopherol, the cut-off level was also higher than 12 μmol/L (*19*), and the ratio to serum total cholesterol below 2.2 indicating a risk of deficiency (*20*) was not found. The values of serum retinol below 0.70 μmol/L have been considered low, and below 0.35 μmol/L have been considered deficient (*21*). Nobody was deficient in this study. The 95% confidence intervals of serum vitamin B_6, blood vitamin B_1, B_2, and blood nicotinic acid were consistent with the previous reports (*10, 19, 22, 23*).

Erythrocytes contain about 90% of blood total vitamin B_1 (*24*). Concentration of vitaminB_1 in whole blood correlated with that in erythrocyte, but did not correlate with that in serum. The results seem that the percentage of vitamin B_1concentration in erythrocyte increase as concentration in whole blood is increasing.

The interactions among blood vitamins were found in antioxidant vitamins. In this study, the negative correlation between serum α-tocopherol and ascorbic acid was found. The result was in contrast with the previous report in supplement users (*25*).

In conclusion, young women had low energy intake and low intake of vitamin E and folate. There were significant correlations between intake of energy and the above mentioned nine vitamins. Therefore physical activities should be increased and the sufficient energy intake corresponding the energy expenditure should be taken for sufficient intake of vitamins. Although the blood levels related to intake for these vitamins, the changes of the blood

vitamin levels were small, as far as these vitamins were only supplied from ordinary dietary intake. However, 95% confidence intervals of blood vitamins were not different from the previous values and few subjects were at the risk of marginal vitamin status. Accordingly, it might be necessary for further studies on appropriate requirements of vitamin E and folate for young women.

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Table 1. Vitamins intakes in female students*

Vitamins		Intake	RDA	% Above RDA
Vitamin A	(μgRE)	727±861	540	46.7
	(IUa)	(2422±2868)	(1800)	
Vitamin E	(mg TE)	7.0±2.4	8.0	28.7
Vitamin B_1	(mg)	0.9±0.4	0.8	68.0
Vitamin B_2	(mg)	1.3±0.9	1.0	78.0
Vitamin B_6	(mg)	1.4±0.6	1.2	54.7
Niacin	(mg NE)	24.0±5.8	13	97.3
Vitamin B_{12}	(μg)	4.9±3.6	2.4	76.0
Folate	(μg)	184±75	200	34.0
Vitamin C	(mg)	138±174	100	54.0

*Mean±SD, n=150

Table 2. Pearson correlations between energy and vitamin intakes *

	Energy	Vitamin A	β-carotene	Retinol	α-tocopherol	Vitamin B_1	Vitamin B_2	Vitamin B_6	Niacin	Vitamin B_{12}	Folate	Vitamin C
Energy	1.000											
Vitamin A	0.338	1.000										
β-carotene	0.167	0.697	1.000									
Retinol	0.357	0.715	0.094 §	1.000								
α-tocopherol	0.486	0.522	0.415	0.395	1.000							
Vitamin B_1	0.618	0.377	0.228	0.305	0.331	1.000						
Vitamin B_2	0.566	0.595	0.244	0.579	0.446	0.718	1.000					
Vitamin B_6	0.292	0.432	0.324	0.332	0.401	0.403	0.401	1.000				
Niacin	0.677	0.484	0.310	0.128	0.505	0.678	0.620	0.504	1.000			
Vitamin B_{12}	0.362	0.389	0.175	0.424	0.418	0.243	0.435	0.280	0.580	1.000		
Folate	0.377	0.535	0.477	0.395	0.449	0.420	0.576	0.380	0.472	0.258	1.000	
Vitamin C	0.208	0.342	0.448	0.057 §	0.333	0.333	0.298	0.381	0.336	0.152 §	0.434	1.000

* All variables are log-transformed.

§ All correlation coefficients except these values are significant.

Table 3. Blood vitamin concentrations in female students

Blood vitamins		Total [n=150]	Above RDA [n]		Below RDA [n]		All above RDAs ¶ [n=3]	All below RDAs § [n=4]
Serum retinol	(μmol/L)	1.49±0.44	1.48±0.44	[70]	1.51±0.43	[80]	1.70±0.71	1.40±22.6
Serum β-carotene	(μmol/L)	1.33±1.20	1.48±1.51	[70]	1.19±0.83	[80]	1.43±0.69	1.31±0.62
Serum α-tocopherol	(μmol/L)	32.0±10.5	32.8±11.0	[43]	31.7±10.4	[107]	40.2±20.5	31.9±7.2
Whole blood total vitamin B_1	(nmol/L)	120±34	123±37	[102]	114±26	[48]	136±29	130±28
Whole blood total vitamin B_2	(nmol/L)	202±37	205±35	[117]	189±40	[33] †	210±41	196±37
Serum total vitamin B_6	(nmol/L)	70.3±58.1	64.0±45.2	[82]	77.9±70.2	[68]	77.0±27.9	46.7±8.4
Whole blood total nicotinic acid	(nmol/L)	39.9±9.8	39.9±9.9	[146]	37.3±2.8	[4]	50.4±31.7	37.3±2.8
Serum vitamin B_{12}	(pmol/L)	447±134	453±135	[114]	427±133	[36]	550±60	386±111
Serum folate	(nmol/L)	17.9±5.2	19.2±5.1	[51]	17.2±5.1	[99] †	21.0±6.1	17.6±5.5
Serum total ascorbic acid	(μmol/L)	66.0±15.1	69.4±14.7	[81]	62.0±14.6	[69] ‡	77.4±7.3	54.2±16.4

* Mean±SD, [n]

† Significantly different from above RDA, $p<0.05$.

‡ Significantly different from above RDA, $p<0.005$.

¶ The subjects with intake of energy and these nine vitamins above the RDAs

§ The subjects with intake of energy and these nine vitamins below the RDAs

Table 4. 95% Confidence intervals for blood vitamins in female students*

Vitamins		95% Confidence intervals (mean±SD)		% Below lower limit †
Serum retinol	(μmol/L)	0.73－2.60	(1.50±0.24)	2.7
Serum β-carotene	(μmol/L)	0.20－4.19	(1.28±0.79)	2.0
Serum α-tocopherol	(μmol/L)	16－55	(32±9)	2.7
Whole blood total vitamin B_1	(nmol/L)	72－175	(118±23)	4.0
Whole blood total vitamin B_2	(nmol/L)	140－275	(205±31)	3.3
Serum total vitamin B_6	(nmol/L)	21－98	(53±17)	2.0
Whole blood total nicotinic acid	(nmol/L)	23－58	(39±8)	0.7
Serum vitamin B_{12}	(pmol/L)	220－760	(443±122)	0.7
Serum folate	(nmol/L)	10－31	(19±4)	4.7
Serum total ascorbic acid	(μmol/L)	42－97	(68±12)	3.3

* Obtained from the subjects with intake above RDA except for β-carotene.

† Percent among all subjects (n=150).

Table 5. Correlations between blood vitamin concentrations in female students*

	Retinol	β-carotene	α-tocopherol	Vitamin B_1	Vitamin B_2	Vitamin B_6	Nicotinic acid	Vitamin B_{12}	Folate	Ascorbic acid
Retinol	1.000									
β-carotene	0.189†	1.000								
α- tocopherol	0.444§	0.286‖	1.000							
Vitamin B_1	0.012	0.095	0.189†	1.000						
Vitamin B_2	0.078	0.162†	0.204†	0.160	1.000					
Vitamin B_6	0.161†	0.086	0.017	-0.027	0.061	1.000				
Nicotinic acid	-0.040	0.024	0.020	0.235¶	0.055	-0.051	1.000			
Vitamin B_{12}	0.055	0.205†	0.237‡	0.126	0.132	0.041	-0.054	1.000		
Folate	0.159	0.212‡	0.161†	-0.043	0.029	-0.088	0.086	0.220‡	1.000	
Ascorbic acid	0.099	0.059	-0.191†	-0.157	-0.136	0.194†	-0.001	-0.011	0.125	1.000

*All vitamins are log-transformed. Vitamin B_1, B_2, and nicotinic acid in whole blood and the others in serum are determined.
†$p<0.05$, ‡$p<0.01$, ¶$p<0.005$, ‖$p<0.0005$, §$p<0.0001$.

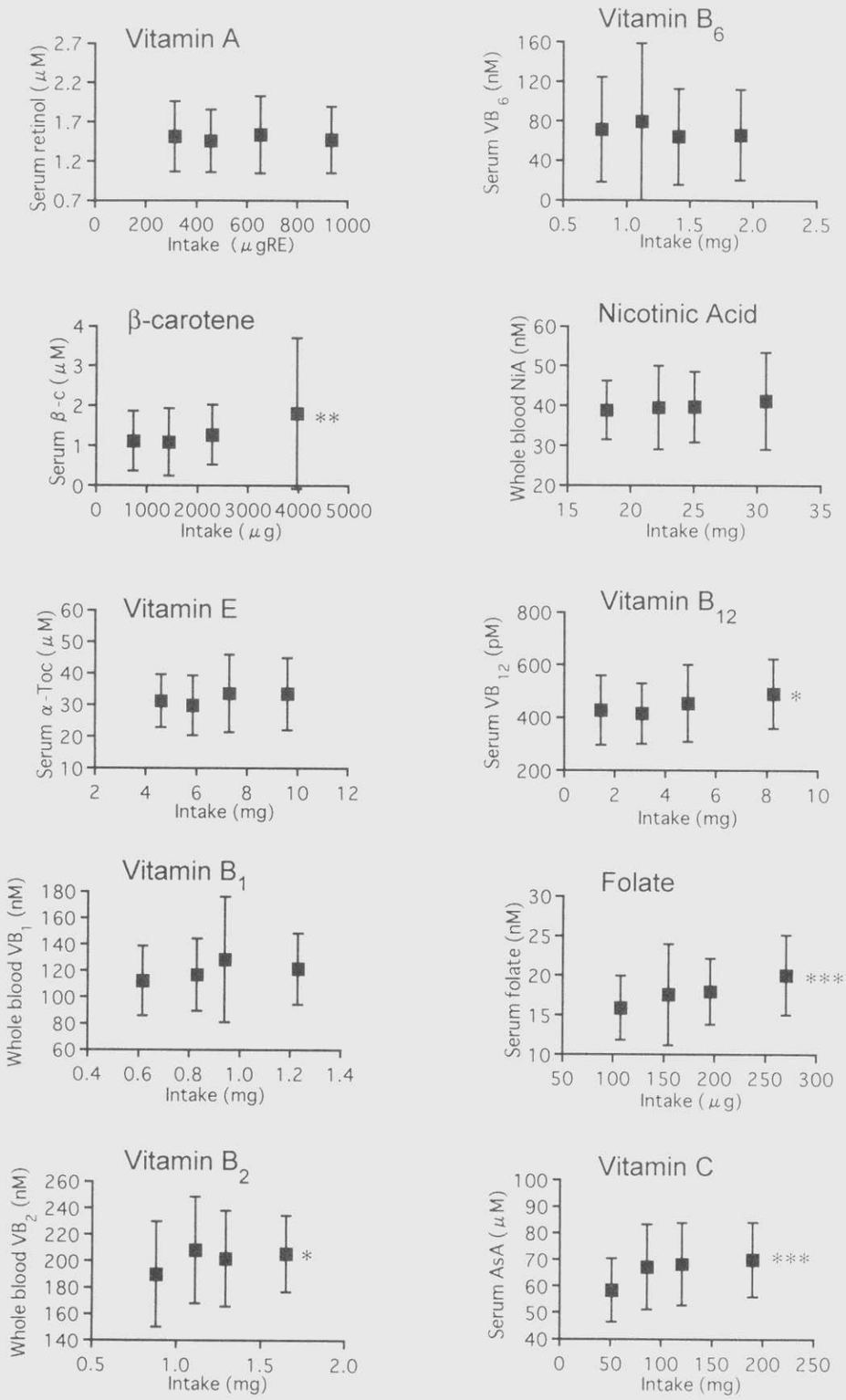

Fig. 1. Mean blood vitamin concentrations (and SD) by quartiles of vitamin intakes. Test for linear trend: p=0.01 for folate, p=0.04 for vitamin C. *p<0.05, **p<0.005, ***p<0.001 for comparisons to mean in the lowest quartile.