J. Food Sci. Nutr.
Vol. 5, No. 4, p. 205~212 (2000)

Biochemical Indices of Vitamin E, Ascorbic Acid and Iron Status : Relation to Diet, Supplement Use and Other Lifestyle Variables in Urban and Rural (Amish) Populations

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Abstract

The relation of food and supplemental intake of iron, vitamin E and ascorbic acid and other lifestyle variables to packed cell volume (PCV) and serum vitamin levels was studied in urban and rural (71% Amish) communities. Subjects were interviewed (24-h dietary recalls) on three occasions over 18-months, and blood samples were taken (maximum observations = 442). Mean PCV was lower in rural males (43.3) than in urban males (45.4) despite higher mean food iron intake (18.7 and 14.4 mg/day, respectively). Mean meal iron availability was higher at lunch and lower at breakfast and dinner for rural than for urban subjects. Smoking was the number one variable in males and females explaining variance in PCV. Supplemental vitamin E and ascorbate intakes explained the most variance in serum vitamin E and ascorbate levels, respectively. Serum vitamin E was also associated with supplemental ascorbate intake (r=0.29). Serum ascorbate was also associated with food ascorbate intake (r=0.28) and body weight (r=-0.24).

Key words: iron, vitamin E, ascorbic acid, packed cell volume

INTRODUCTION

The nutritional status of groups of Americans and its relation to nutrient intake and other lifestyle variables is of continuing concern to health specialists and the frequent subject of nationwide as well as limited local dietary surveys. In the 1965 Nationwide Food Consumption Survey the mean dietary intake of three minerals including ascorbic acid was lower than the 1968 RDA (1) for one or more sex-age groups (2). In the 1977~78 Nationwide Food Consumption Survey (2) and the Second Health and Nutrition Examination Survey (HANES 2) (3) mean intakes of vitamin C exceeded the 1980 RDA (4) for all sex-age groups, but iron intakes provided as little as 53 percent of the 1980 RDA in some groups. Recently the National Nutrition Monitoring System identified shortfall nutrients as iron, Ca, Zn, vitamin A, vitamin B_6 and vitamin C (5). Due to deficiencies in food composition information, intakes of many nutritients including vitamin E have not been studied in the surveys (2), but in an analysis of menus based on the UDSA Thrifty Food Plan or the Four Food Group Plan, vitamin E levels were below the RDA (6,7).

In HANES 2 (8) approximately 3.6% of the study population had less than acceptable serum ascorbic acid levels (<14 g/dL, males; <12 g/dL, females). Presumably, biochemical measurements not subject to homeostatic control are more sensitive indicators of nutritional status than dietary information (9,10). However, biochemical indices of nutritional status and dietary intake information have not been correlated in at least one national nutritional survey (11). Certainly, difficulties associated with accurate measurements of nutrient intakes and individual differences in nutrient requirements could account for the weak associations. In some instances attitudinal behavioral variables may be more predictive of nutritional status than intake variables (9,12).

Ascorbic acid is known to be the most effective water soluble dietary antioxidant. It appears to protect against lipid peroxidation by scavenging peroxyl radicals in the aqueous phase before they can initiate lipid peroxidation and by regenerating the active form of vitamin E, lipophilic antioxidant. Futhermore ascorbic acid is involved in the regulation of iron metabolism (13-15). It increases absorption of nonheme iron and iron deficiency is a significant public health problem in all social strata, not just among the poor in USA (16-19).

In this study we investigated the association of nutrient intake and other selected lifestyle variables with biochemical indices of vitamin E, ascorbic acid and iron status in two sample populations with very different lifestyles. One was a group of middle class urban subjects living in a consumer test market (Columbus, Ohio). The other group consisted of rural, predominately Amish, subjects who had a lifestyle and diet more typical, we thought, of early 20th century America.

MATERIALS AND METHODS

Subjects and experimental design

Rural and urban subjects recruited for this prospective

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study were interviewed by trained nutritionists on a maximum of three occasions, 4 to 6 months apart. Each interview occurred on a different day of the week in a different season of the year. Casual venous blood samples were drawn into vacutainers with heparin or without anticoagulant at the time of or within ten days of each interview. All subjects, or in the case of minor children, subjects' parents, signed an informed consent form approved by the Ohio State University Biomedical Sciences Human Subjects Review Committee prior to the first interview.

The intial rural sample consisted of 73 males and females (52 Old Order Amish: 10 Mennonite), ranging in age from 5 to 73 years. Most rural households were actively engaged in farming and grew much of their own food. Eighty-two subjects, 4 to 76 years of age, comprised the initial urban sample. These subjects lived in middle to upper class neighborhoods and purchased most of their food at supermarkets or restaurants. Only subjects completing three interviews were included in the studies of vitamin E and iron. Serum ascorbate data were more limited due to the fact that blood samples for ascorbate analyses were not drawn during the early interviews and were not obtained for subjects under 20. All subjects providing one or more blood samples for ascorbic acid analysis were used in the ascorbic acid study.

Dietary data were obtained by 24-h dietary recall. Information on nutrient supplement use, alcohol, caffeine and cigarette consumption, and on energy expenditure in daily physical activities was also obtained during the interviews.

Intake analyses

Each dietary recall was coded and analyzed by the Ohio State University Hospital Dietary department Nutrient Data Base for intakes of ascorbic acid, iron, food energy and the other nutrients used as independent variables in the multiple regressions analyses. Since dietary information for vitamin E is incomplete in The Ohio State University Hospital Data Base, vitamin E intakes were computed manually using food labels and a table of alpha tocopherol values (20). Alpha tocopherol intakes (mg) were multiplied by 1.2 to compute total vitamin E activity in milligrams alpha tocopherol equivalents (4). Supplemental vitamin E intake is reported, as it was on the supplement labels, as International Units. The alcohol and caffeine intakes used in the regression analyses, were also computed manually (21,22).

The Ohio State University Nutrient data Base also has the capacity to compute the proportion of nutrient intake provided by 22 different food groups. Using this option we identified the food groups providing the greatest percentages of ascorbate intake for rural and urban population samples. We also used the option to compute the proportion of iron intake provided by animal flesh foods and from this information calculated heme iron intake (40 percent of total iron of animal tissues (16). The mean availability of iron in meals consumed by rural and urban subjects was calculated from the meat, poultry, fish, and ascorbic acid content of the meal using published procedures (4). High, medium and low availability meals were assigned values of 3, 2, and 1, respectively (23).

Biochemical analyses

Blood samples were packed in ice, transported to the laboratory and cells were separated within 8 h (typically 4 h). Packed cell volumes were determined in duplicate. For ascorbic acid analyses, serum samples were deproteinized with four volumes of 5% trichloroacetic acid and were stored at -20°C until analysis with 2,4 dinitrophenylhydrazine (24). Results of a prestudy indicated that ascorbate activity remained stable during this procedure. A colorimetric assay was used to determine plasma vitamin E concentration (25).

Statistical analyses

Statistical analysis system (SAS) procedures were used to compute least squares means and correlations for the iron and vitamin E studies. The population samples were divided into subgroups by location and gender, and least-squares means were adjusted for unequal subclass frequencies with respect to location, gender, location by gender, age and weight. Harvey's Mixed Model Least Squares and Maximum Likelihood Computer Program (26) was used to compute correlations and least squares means adjusted for a variety of variables including location, gender, location by gender, age, weight, and interview for the ascorbate data. For step-wise multiple regression analyses, data points obtained at the interviews were averaged to give one value per subject for each variable of interest. Subjects under 20 were not included in the analyses. Packed cell volume, and serum ascorbate and vitamin E concentrations were the dependent variables. The statistical model included the following independent variables : absolute age; age group (20~45 or over 45); gender; body weight; relative body weight (according to the 1959 Metropolitan Life Insurance tables (27); daily intake of food and supplemental iron, vitamin E and ascorbic acid; daily intake of energy, protein, carbohydrate, fat, cholesterol. linoleic acid, saturated fat and selenium; cigarettes smoked per day; number of years of cigarette smoking; cups of coffee equivalents consumed per day; alcoholic drinks consumed per month; and kcal expended per kg body weight in daily activities. Regression analyses were run for the entire cohort, males, females and urban and rural subjects.

RESULTS

Iron intake and packed cell volume

In this study rural subjects consumed significantly more food iron but had significantly lower packed cell volumes (Table 1). Rural males tended to consume more food and supplemental iron than urban males. On the average, urban females consumed more supplemental iron than rural females but similar amounts of food iron. Thus, there was a significant location : gender interaction for food and supplemental iron intake. The range of food and supplemental iron intakes as determined at individual interviews was large for all location

Table 1. Least-squares means±SEM and ranges of supplemental and food iron intakes and packed cell volumes[1)]

Group	n	Iron intake (mg/day)		Packed cell volume	
		Food	Supplemental	n	Percent
Location					
Rural	213	15.3±0.5	3.5±0.8	213	41.2±0.2
Uuban	299	13.0±0.4	4.0±0.8	225	43.5±0.2
P		0.0003	0.6609		0.0001
Gender					
Males	214	16.6±0.5	2.3±0.8		44.3±0.2
Females	228	11.7±0.5	5.3±0.8		40.4±0.2
P		0.0001	0.0129		0.0001
Location × Gender					
Rural males	108	18.7±0.6 (5.5-57.0)	3.3±1.1 (0-60.0)	108	43.3±0.3 (34.5-51.2)
Rural females	105	11.9±0.7 (1.2-31.1)	3.9±1.1 (0-60.0)	105	39.1±0.3 (29.0-48.0)
Urban males	106	14.4±0.7 (2.7-38.8)	1.4±1.2 (0-27.0)	105	45.4±0.3 (37.2-52.2)
Urban females	123	11.5±0.6 (3.2-40.2)	6.7±1.1 (0-65.0)	120	41.8±0.3 (32.0-47.0)
P (interaction)		0.0049	0.0420		0.3973

[1)]Least-squares means adjusted for unequal subclass frequencies with respect to location, gender, location by gender, age and weight (General Linear Models Procedure)

: gender groups (Table 1). This was true for all nutrients studied in this project. The following mean percentages of the RDA were provided by food iron : rural males, 180±6; rural females, 80±6; urban males, 142±7; and urban females, 80±7. When supplements were included in the intake, mean intake for these population samples ranged from 105 (rural females) to 213 (rural males) percent of the RDA. The range of packed cell volumes suggested that some subjects had less than acceptable values. The latter vary with age and gender, for example, <44 for adult males and <33 for adult females (28). Numbers of subjects displaying less than acceptable average values for all blood tests were as follows: rural males, 11; rural females, 8; urban males, 5; and urban females, 3. None of the subjects had "deficient" values for all tests.

The possibility was investigated that rural subjects, especially males, consumed diets with lower iron bioavailability. However, there was no significant difference in heme iron intake between rural and urban subjects (Table 2), but females consumed significantly less heme iron than males. The iron bioavailability of the breakfast and dinner meals was significantly higher for urban subjects than rural subjects while that of the "lunch" meal was significantly lower. An explanation was that the midday meal of the rural subjects tended to be a heavy meal with more meat whereas the evening meal was lighter. Rural subjects tended to consume less meat and vitamin C sources at breakfast than urban subjects. There was

Table 2. Least-squares means±SEM of heme iron intakes and bioavailability of iron in meals[1)]

Group	n	Heme iron intake (mg/day)	Iron bioavailabililty[2)]		
			Breakfast (n)	Lunch (n)	Dinner (n)
Location					
Rural	196	1.6±0.1	1.30±0 05 (204)	2.04±0.06 (209)	1.97±0.06 (203)
Urban	218	1.6±0.1	1.52±0.05 (205)	1.81±0.06 (219)	2.31±0.05 (227)
P		0.9448	0.0023	0.0042	0.0001
Gender					
Males	202	1.8±0.1	1.41±0.05 (192)	2.02±0.06 (207)	2.22±0.06 (209)
Females	212	1.3±0 1	1.41±0.05 (217)	1.83±0.06 (221)	2.06±0.06 (221)
P		0.0010	0 9591	0.0285	0.058
Location×Gender					
Rural males	103	1.8±0.1	1.32±0.07 (104)	2.16±0.08 (106)	2.06±0.08 (105)
Rural females	93	1.3±0.1	1.29±0.07 (100)	1 93±0.08 (103)	1.88±0.08 (98)
Urban males	99	1.7±0.1	1.50±0.08 (88)	1.89±0.09 (101)	2.38±0.08 (104)
Urban females	119	1.4±0.1	1.53±0.07 (117)	1.74±0.08 (118)	2.24±0.08 (123)
P (interaction)		0.3121	0.7038	0.6227	0.819

[1)]Least-squares means adjusted for unequal subclass frequencies with respect to location, gender, location by gender, age and weight (General Linear Models Procedure)

[2)]High, medium, and low iron availability meals are rated 3, 2, and 1, respectively. See methods section for calculation details.

no significant location : gender interaction in heme iron intake or bioavailability of iron in meals. The iron bioavailability of midday and evening meals tended to be lower for females than for males probably due to lower meat consumption.

Results of correlation and multiple regression analyses shed some light on factors associated with packed cell volumes of adult subjects in this study (Tables 3, 4). Thirteen variables in the model had significant Pearson correlations with packed cell volume, the strongest of which, not surprisingly, was gender (-0.64), which accounted for 43 percent of the variance in PCV in the entire cohort (Table 4) in multiple regression analysis. The factor appearing most consistently in multiple regression analyses was cigarette smoking, the number one variable in analyses for males and females (Table 4). Age or age group also appeared consistently in all analyses involving females. Food but not supplemental iron intake was significantly correlated with packed cell volume in the entire cohort (Table 3) but only appeared in the multiple regression analyses for rural subjects (not shown) where it explained about 2 percent of the variance.

Table 3. Significant ($p<0.05$) Pearson correlation coefficients for packed cell volumes and selected independent variables (entire cohort)[1)]

Independent variable	r (n=124)	p
Gender	-0.64	0.0001
Protein intake	0.42	0.0001
Food energy intake	0.37	0.0001
Selenium intake	0.36	0.0001
Fat intake	0.34	0.0001
Body weight	0.33	0.0002
Iron intake	0.32	0.0003
Cigarettes/day	0.32	0.0003
Relative body weight	0 31	0.0008
Carbohydrate intake	0.29	0.0010
Linoleic acid intake	0.28	0.0019
Saturated fat intake	0.23	0.0099
Alcohol intake	0.21	0.0195

[1)]Average subject value for each variable used in computing correlations (adults only).

Vitamin E intake and serum E levels

Rural subjects consumed significantly more supplemental vitamin E, less food E, and had higher serum vitamin E levels than urban subjects (Table 5). Food vitamin E provided 84 ± 7.5 and 100 ± 5 percent of the RDA for rural and urban respondents, respectively. Some rural subjects, especially males, consumed massive doses of supplemental vitamin E ranging

Table 4. Stepwise multiple regression analyses of packed cell volumes and selected independent variables[1)]

Variable	R^2	β
Entire Cohort (n=114)		
Intercept		41.0543
Gender (males=1; females=2)	0.44	4.0344
Cigarettes/Day	0.50	0.1282
Age group (20-45=1;.45=2)	0.52	1.2834
Protein intake (g/day)	0.53	0.0167
Males (n=56)		
Intercept		44.6910
Years of smoking	0.12	0.07910
Females (n=58)		
Intercept		34.7170
Cigarettes/Day	0.16	0.2477
Age group (20-45=1;.45=2)	0.33	5.7454
Age (years)	0.40	-0.1219
Protein intake (g/day)	0.43	0.0746
Fat intake (g/day)	0.47	-0.0374

[1)]Average subject value for each variable used in multiple regression (adults only).

Table 5. Least-squares means ± SEM and ranges of supplemental and food vitamin E intakes and serum vitamin E concentrations[1)]

Group	n	Vitamin E intake		
		Food (mg α-tocopherol equiv.)	Supplemental (IU)	Vitamin E (μg/mL)
Location				
Rural	210	7.4 ± 0.4	180.7 ± 29 4	13.4 ± 0.3
Urban	219	8.8 ± 0.4	24 4 ± 28.8	12.0 ± 0.3
P		0.0018	0.0002	0.0011
Gender				
Males	210	8.8 ± 0.4	128.2 ± 30.9	12.4 ± 0.3
Females	219	7.4 ± 0.4	76.8 ± 30.1	12.9 ± 0.3
P		0.0221	0 2541	0.2671
Location × Gender				
Rural males	105	8.4 ± 0.6 (0.1-34.0)	215.9 ± 41.7 (0-4571)	12.9 ± 0.4 (6.8-32.4)
Rural females	105	6.4 ± 0.6 (0.1-29.4)	145.4 ± 41.7 (0-1800)	13.9 ± 0.4 (6.6-41.1)
Urban males	105	9.2 ± 0.64 (0.9-33.2)	0.6 ± 44.8 (0-415)	12.0 ± 0.5 (5.1-21.7)
Urban females	114	8.4 ± 0.6 (2.0-45.3)	8 1 ± 42.4 (0-800)	12.0 ± 0.4 (5.8-29.7)
P (interaction)		0.3001	0.6599	0.2485

[1)]Least-squares means adjusted for unequal subclass frequencies with respect to location, gender, location by gender, age and weight (General Linear Models Procedure)

up to 4571 IU (3068 mg alpha tocopherol equivalents) per day. Supplemental vitamin E had the strongest Pearson correlation (p=0.51) with plasma vitamin E of any of the variables studied (Table 6) and was the number one variable in the multiple regression analyses for the entire cohort, males and females, explaining 26, 42, and 20 percent of the variance, respectively (Table 7). Age or age group was also consistently associated with plasma vitamin E levels (Table 6, 7). Supplemental ascorbate was positively associated with plasma vitamin E in some analyses (entire cohort, males, rural subjects), explaining from 3 up to 10 percent of the variance. Food vitamin E intake was generally not an important factor in these analyses, but it did explain about 3 percent of the variance in plasma E in rural subjects.

Ascorbic acid intake and serum ascorbate

Rural and urban subjects did not differ significantly in mean serum ascorbate concentration or in mean intake of food or supplemental ascorbate although there was a tendency for rural subjects to consume more supplement (Table 8). The mean intake of food ascorbate ranged from 1.53 up to 2.35 times the RDA (60 mg). Rural males tended to consume more ascorbate than rural females. Nevertheless, females had a sighificantly higher mean serum ascorbate level than males. None of the subjects had deficient or even low (<0.4 mg/dL) (29) serum concentrations of ascorbate.

Since rural subjects, particularly the Amish, were more likely to eat home-grown foods and were less likely to purchase citrus fruits (not grown in Ohio) than urban subjects, it was of interest to determine the significant food sources of ascorbic acid for the two population samples. Ascorbate-rich fruits provided over one-third of the ascorbate intake of urban subjects (Table 9), which is agreeable to 38.6%

Table 6. Significant (p<0.05) Pearson correlation coefficients for serum vitamin E and selected independent variables (entire cohort)[1)]

Independent variable	r (n=124)	p
Supplemental vitamin E	0.51	0.0001
Age group	0.33	0.0002
Age	0.32	0.0003
Supplemental ascorbate	0.29	0.0011

[1)]Average subject value for each variable used in computing correlations (adults only).

Table 7. Stepwise multiple regression analyses of plasma vitamin E and selected independent variables[1)]

Variable	R^2	β
Entire Cohort (n=114)		
Intercept		6.4110
Supplemental vitamin E (IU/day)	0.26	0.0039
Age (years)	0.32	0.0781
Supplemental ascorbate (mg/day)	0.36	0.0033
Gender (1=male; 2=female)	0.38	1.8886
Food selenium (ug/day)	0.40	0.0167
Males (n=56)		
Intercept		6.8309
Supplemental vitamin E (IU/day)	0.42	0.0033
Supplemental ascorbate (mg/day)	0.52	0.0036
Age (years)	0.57	0.0733
Fat intake (g/day)	0.59	0.0135
Females (n=58)		
Intercept		7.5200
Supplemental vitamin E (IU/day)	0.20	0.0070
Age (years)	0.26	0.0648
Food iron intake (mg/day)	0.30	0.2129

[1)]Average subject value for each variable used in multiple regression analyses (adults only).

Table 8. Least-squares means±SEM and ranges of supplemental and food ascorbate intakes and serum ascorbate concentrations[1)]

Group	Ascorbate intake (mg/day)				Serum ascorbate	
	n	Food	n	Supplemental	n	mg/day
Location						
Rural	174	117±9	171	166±45	140	1.44±0.05
Urban	204	125±8	204	76±39	192	1.37±0.04
P		0.4942		0.1187		0.2644
Gender						
Males	185	134±8	182	125±33	164	1.33±0.04
Females	193	108±8	193	117±32	168	1.48±0.04
P		0.0012		0.7543		0.0021
Location×Gender						
Rural males	90	141±10	87	174±39	74	1.39±0.05
		(3-386)		(0-1575)		(0.46-2.22)
Rural females	84	92±11	84	157±40	66	1.49±0.06
		(5-281)		(0-1200)		(0.42-2.42)
Urban males	95	127±10	95	76±35	90	1.27±0.05
		(5-405)		(0-1200)		(0.41-2.26)
Urban females	109	123±9	109	76±34	102	1.48±0.04
		(1-421)		(0-560)		(0.43-2.54)
P (interaction)		0.0047		0.7561		0.1902

[1)]Least-squares means adjusted for unequal subclass frequencies with respect to location, family/location, gender, location by gender, gender by family/location, interview, location by interview, gender by interview, age and weight (Harvey's Mixed Model and Maximum Likelihood Computer Program)

Table 9. Least-squares means±SEM of percentages of ascorbate intake coming from selected food group[1)]

Food group	Location		p
	Rural (n=174)	Urban (n=204)	
Ascorbate-rich fruits	24.2±0.2	35.3±0.2	0.0093
Vitamin A-rich vegetables	4.4±1.5	5.9±1.4	0.4531
Other fruits	12.5±1.6	10.7±1.5	0.4252
Milk	6.2±1.3	6.7±1.2	0.7894
Cereals	6.5±1.0	3.8±0.9	0.0527
Other vegetables	25.8±2.0	21.5±1.9	0.1188
Other beverages	2.7±1.2	3.0±1.0	0.8488
Desserts	7.1±0.8	1.9±0.8	0.0000

[1)]Least-squares means adjusted for unequal subclass frequencies with respect to location, family/location, gender, location by gender, gender by family/location, interview, location by interview, gender by interview, age and weight (Harvey's Mixed Model and Maximum Likelihood Computer Program)

of dietary vitamin C from citrus fruits and juices in other study (30) but only one-forth of the rural intake, a significant difference. A major source of ascorbate for rural residents was other (than vitamin A rich) vegetables. This type of vegetable was likely to be home grown. Desserts and cereals also contributed more ascorbate to the diet of the rural people than the urban residents.

Supplemental ascorbate was the most important factor in the statistical model associated with serum ascorbate (Table 10, 11). Food ascorbate was also consistently associated with higher serum ascorbate levels in all correlation and multiple regression analyses performed. Interestingly, body weight was associated with lower level of serum ascorbate in the entire cohort and in males. Female gender was associated with higher levels.

DISCUSSION

Nutrient intake and status

Daily food intake of iron, vitamin E (mg alpha tocopherol equivalents) and ascorbic acid of rural and urban subjects varied widely from day to day from less than one milligram up to as much as seven times the RDA. Mean daily intakes of food ascorbate, over twice the RDA for all population samples except rural females, ranged between the 50th and the 75th percentile of intake estimated for HANES II respondents (3). Fifty of the subjects regularly ingested supplemental ascorbate of sufficient potency in some cases that the mean total intake of both rural and urban samples exceeded 200 mg per day.

Table 10. Significant ($p<0.05$) Pearson correlation coefficients for serum ascorbate and selected independent variables (entire cohort)[1)]

Independent variable	r (n=124)	p
Supplemental ascorbate	0.33	0.0002
Food ascorbate	0.28	0.0015
Gender	0.25	0.0057
Weight	-0.24	0.0062
Fat intake	-0.20	0.0300
Energy intake	-0.18	0.0414

[1)]Average subject value for each variable used in computing correlations (adults only).

Table 11. Stepwise multiple regression analyses of serum ascorbate and selected independent variables[1)]

Variable	R^2	β
Entire cohort (n=114)		
Intercept		1.3744
Supplemental ascorbate (mg/day)	0.11	0.0004
Weight (lbs)	0.21	-
Food ascorbate (mg/day)	0.29	0.0021
Gender replaces weight (1=male; 2=female)	0.32	0.1395
Food vitamin E (mg/day)	0.34	-0.0147
Weight	0.36	-0.0017
Males (n=56)		
Intercept		1.9830
Supplemental ascorbate (mg/day)	0.19	0.004
Food ascorbate (mg/day)	0.35	0.0025
Weight (lbs)	0.46	-0.0047
Food vitamin E (mg/day)	0.54	-0.0251
Saturated fat intake (g/day)	0.60	-0.0085
Protein intake (g/day)	0.80	0.0029
Females (n=58)		
Intercept		1.2105
Supplemental ascorbate (mg/day)	0.07	0.0005
Food ascorbate (mg/day)	0.17	0.0017

[1)]Average subject value for each variable used in multiple regression analyses (adults only).

Mean food iron intakes of all males and females in this study (161 and 80 percent of the RDA, respectively) were in agreement with means estimated for all males and females above poverty level in HANES II (8) but higher than that for women aged 18 to 44 years in other study (31). The mean total iron intake of all rural and urban females exceeded the RDA, although this might not have been the case for all women of child-bearing age.

Mean intakes of food vitamin E were 10~20 percent below the RDA for all population samples except urban females. Fifty three of the subjects took vitamin E supplements of such potency in some cases that mean total supplemental intake exceeded the RDA considerably in all population samples except urban females. Mean supplemental intake of rural males was almost 10 times the RDA.

According to their serum chemistries all subjects were in adequate ascorbate and vitamin E status at all times during the 1.5 year study. This finding was in contrast to HANES II in which 3.6 percent of the population had unacceptable serum ascorbate values (8). Mean serum ascorbate values for males and females in this study were 0.2~0.3 mg/dL higher than corresponding values for HANES II respondents. As in HANES II, females in this study had higher serum ascorbate levels than males although they consumed somewhat less ascorbic acid on the average. This finding suggests that

females, perhaps because of lower body weight, need less of this vitamin than males, a viewpoint reflected in an earlier edition of the RDA. Body weight had a significant negative correlation with serum ascorbate levels in the entire cohort and explained about 10 percent of the variance in serum ascorbate in the entire cohort and in males and 17 percent of the variance in urban respondents (data not shown).

Packed cell volumes were 1~1.5 percentage points higher for men and women in this study than in HANES II (8), where the mean value for males of all ages and races (42.8 percent) was in the marginal category according to the criterion used for adult males in the 10 State Nutrition Survey (28). About 18 percent of the subjects in this study had low but not deficient packed cell volumes. More low values were noted in rural than in urban subjects. Nevertheless, it is obvious that the numerous pregnancies occurring in rural female respondents, who commonly had more than six and sometimes as many as eleven children, did not result in serious anemia at least in our sample. But lower packed cell volumes is troublesome and will be discussed further in the next section.

Lifestyle variables and serum chemistries

Currently, there is considerable interest in identifying biochemical markers, such as level of a nutrient in blood, that will give an accurate indication of past nutrient intakes (29). Reliable biochemical markers should not be under homeostatic control and should be highly associated with nutrient intake.

In this study the only serum chemistry consistently associated with recent past food intake was serum ascorbate. However, supplemental ascorbate and supplemental vitamin E were the most important variables identified in this study as being associated with serum ascorbate and serum vitamin E, respectively. Schutz (12) also observed that vitamin supplement use and daily vitamin C intake were predictive of serum vitamin C levels. Johnson et al. (32) indicated that factors affecting serum ascorbate levels in HANES II included age, gender, smoking and vitamin supplement use. Smokers metabolize vitamin C rapidly and have lower ascorbate levels in blood than nonsmokers at equal intakes (33). Smoking was not predicitive of serum ascorbate levels in this study.

There have been few, if any, studies of variables associated with serum vitamin E levels. The relatively consistent and positive association in this study of age and serum vitamin E level probably was related to supplement use, the number one variable in the multiple regression analyses for serum vitamin E. Younger respondents did not take large doses of vitamin E and tended not to take supplements at all. The positive and consistent association of supplemental ascorbate with serum vitamin E was interesting and merits further investigation since both nutrients act as antioxidants. The relationship of serum vitamin E to selenium intake was modest at best. Selenium intake was not correlated with serum vitamin E but explained 2 percent of the variance the entire cohort.

One of the most interesting observations made in this study was that rural males consumed on the average more food and supplemental iron than urban males yet had a mean lower packed cell volume. Even though packed cell volume is not a conclusive measure of iron status (28), food iron intake was positively and significantly correlated with packed cell volume in the entire cohort and explained a small amount of the variance in this parameter in the rural cohort. Since the rural and urban populations differed considerably in lifestyle, we considered the possibility that differences in the various lifestyle correlates would explain the results we obtained with rural and urban males. Although over half of the independent variables we studied had significant and positive univariate correlations with packed cell volume, the most important variable, other than gender, appearing in the multivariate analyses was smoking, which was associated with higher packed cell volumes. The fact that rural, particulary Amish, males tended not to smoke may explain their lower packed cell volumes despite their higher mean iron intake.

Singer et al. (34), as a result of an analysis of HANES I data on women 12~54 years of age, claimed that demographic variables such as poverty index ratio, education, age, race, oral contraceptive use, pregnancy and previous diagnosis of anemia were associated with variance in hemoglobin levels. Food iron intake was only associated with hemoglobin level in children 1~3 years of age. Employment status and seven consumer attitudes were more strongly associated with iron binding capacity of the blood than was iron intake in 100 adult women (12). We must concur, therefore, with the numerous other pieces of evidence indicating that indices such as packed cell volume, hemoglobin, and iron binding capacity are not highly sensitive indicators of iron intake, at least in subjects that are not so deficient in iron that they have deficient values for these parameters.

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(Received August 12, 2000)